

## CHAPTER 6 - EXPOSURE OF SELECTED POTENTIAL ENDOCRINE DISRUPTING CHEMICALS IN HUMANS AND WILDLIFE ..... 2

6.1	Introduction.....	2
6.2	General exposure issues.....	5
6.2.1	Sources .....	6
6.2.2	Exposure pathways .....	6
6.2.2.1	Air.....	7
6.2.2.2	Water .....	7
6.2.2.3	Soil .....	8
6.2.2.4	Sediment.....	8
6.2.2.5	Food.....	9
6.2.2.6	Consumer products .....	10
6.2.3	Intake and uptake .....	11
6.2.4	Internal Dose and Pharmacokinetics .....	11
6.3	Case studies.....	12
6.3.1	Wildlife exposures .....	12
6.3.1.1	Persistent, lipophilic EDCs.....	13
6.3.1.1.1	The Baltic Sea .....	13
6.3.1.1.2	The Great Lakes .....	15
6.3.1.1.3	The Arctic Region.....	17
6.3.1.1.4	Global distribution of DDT and PCB compounds in marine mammals .....	18
6.3.1.2	Tributyl tin .....	19
6.3.1.3	Alkylphenols and ethoxylates and their degradation products (APEs) .....	21
6.3.1.4	Summary and conclusions of wildlife exposures .....	25
6.3.2	Human exposures – some selected case studies .....	25
6.3.2.1	“Dioxins” .....	26
6.3.2.2	Polybrominated diphenyl ethers (PBDEs).....	29
6.3.2.3	DDT .....	30
6.3.2.4	Phthalates .....	31
6.3.2.5	Atrazine.....	32
6.3.2.6	Phytoestrogens.....	32
6.3.2.7	Conclusions on human exposures .....	33
6.4	Measurement of exposure to EDCs.....	34
6.4.1	Sampling .....	34
6.4.2	Analytical considerations .....	35
6.4.2.1	Measuring specific chemicals .....	35
6.4.2.2	Identifying unknown samples .....	35
6.4.2.2.1	Biologically-Based Methods.....	36
6.4.2.2.2	Toxic identification and evaluation approach (TIE) .....	37
6.4.3	Mixtures .....	38
6.4.3.1	Chirality considerations .....	38
6.4.3.2	TEF/TEQ Approaches .....	39
6.4.4	Quality assurance/quality control.....	39
6.4.5	Exposure models.....	40
6.4.6	Structure Activity Relationships .....	41
6.5	Summary .....	42

## **CHAPTER 6 - EXPOSURE OF SELECTED POTENTIAL ENDOCRINE DISRUPTING CHEMICALS IN HUMANS AND WILDLIFE**

### **6.1 Introduction**

The availability of validated exposure data is a critical component for assessing the causal relationships between exposure to endocrine-disrupting chemicals (EDCs) and health effects. Previous chapters examined the state-of-the-science regarding potential effects of EDCs in wildlife and humans. This chapter focuses on exposure issues and analytical approaches and methodologies particularly relevant to EDCs, by using some illustrative case studies in wildlife and humans. It is not intended to evaluate all data published on exposures to potential EDCs. Knowledge about the magnitude or patterns of human and wildlife exposure to EDCs is limited. Current available exposure information has focused mainly on concentrations of persistent organic pollutants (POPs) in Europe and North America. There is limited information on exposures in developing countries and on the less-persistent EDCs. POPs have been transported all over the world, even in regions where they have never been used. There may be considerable redistribution of POPs from warmer to cooler climates (de March et al., 1998). Even though a number of POPs are no longer produced, they have remained in the environment for many decades, and may continue to be unintentionally produced during some industrial processes. A large amount of data was compiled and evaluated for this chapter. This data has been summarized in Annex I. Most of this data, however, was not generated specifically for assessing relationships between exposure to EDCs and adverse health outcomes. In only a few cases have such relationships been clearly established (see Chapters 4, 5 and 7).

Exposure studies aim to determine the nature and extent of contact with a chemical under different conditions, and involve both external measurements (e.g., levels in air, water, food) and internal measurements (e.g., levels in blood, urine, breast milk). Generally, approaches include indirect and direct techniques, measurements of environmental or tissue concentrations, questionnaires, personal monitoring devices, as well as biomarkers and mathematical models (US EPA, 1998; Paustenbach, 2000; IPCS, 2000). Some knowledge of the sources, fate and transport of a chemical and its transformation or degradation in specific media and/or species is required. Emphasis is placed on assessing the magnitude, duration, and frequency of exposure as well as on estimating the number of individuals involved. Comprehensive exposure studies are very costly, and are often constrained by limited resources and ethical considerations. Therefore, priorities

must be set and the purpose must be clearly defined. Reasons for undertaking exposure assessments include epidemiology or field studies, status and trend determinations, and for risk assessment analysis or risk management purposes.

In this document, EDCs have been defined as “exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub) populations. The diversity of chemicals includes natural and synthetic hormones, phytoestrogens, pesticides, and a variety of industrial chemicals and by-products. This enormous diversity means that it is not possible to define a “typical” EDC, and each case must be carefully evaluated as to what chemical(s) to measure, in what matrix, or biological tissue. In addition to their structural diversity, EDCs possess a range of different physicochemical characteristics. Some are persistent, some lipophilic, sequestered in adipose tissue and secreted in milk; while others are hydrophilic and rapidly degraded and may only be present for short periods of time but at critical periods of development. Some complex mixtures (e.g., sewage waste treatment, industrial effluents) contain “hormonally active” components, but the specific chemical entity has not been fully characterized. The ubiquitous presence of natural hormones and plant estrogens pose difficult analytical issues since these natural EDCs may be more potent than environmental EDCs.

In general, exposure studies for EDCs are similar to that for other toxic chemicals. There are, however, some issues which require special emphasis including the diversity of chemicals reported to be hormonally active, the timing and duration of exposure and the general inadequacy of the information currently available on exposure to EDCs.

One of the most important issues which complicate assessing exposure to EDCs in both wildlife and humans is the level, timing, and duration of exposure relative to the developmental stage of the organism (see Chapter 2). Exposure during fixed time frames in development when programming of the endocrine system is occurring may result in permanent changes; whereas exposure during “non-programming” time periods may not result in any significant or detectable effect (see Chapter 3). For wildlife, critical periods of development may include *in utero* or *in ovo* exposures, exposure at different stages of the lifecycle, or exposure at different stages of the reproductive cycle which may have a strong seasonal element. Different life stages may occur in different environments (e.g., the eggs of some insects develop in aquatic environments, whereas, adults are terrestrial) and thus are subject to different exposures. In certain wildlife species, pups

exposure to biomagnified EDCs in the fat rich mother's milk (in seals the fat content may exceed 70%) may consume much higher levels of contaminants per kilogram body weight during the lactation period than during later life stages. It is evident that exposure to EDCs during the fetal or equivalent developmental phase in mammals, birds or fish is of major importance (see Chapter 4 and 5). For example, exposure to PCDDs/PCDDFs and dioxin-like PCBs have been linked to reproductive impairment and teratogenic effects in colonial, fish-eating birds of the Great Lakes (Bowerman et al., 1995; Helander et al., 1999a; Tillitt et al., 1989; Tillitt et al., 1992; Yamashita et al., 1993), and to reproductive and immune dysfunction among Baltic seals (Bergman, 1999). Exposure to nonylphenol during smoltification in Atlantic salmon (Fairchild et al., 1999) can cause adverse effects. In fish, periods of susceptibility occur during fin development which may be greatly influenced by retinoic acid exposure (Vandersea et al., 1998). Retinoic acid is a known vertebrate teratogen (La Clair et al., 1998) and has also been shown to be important to metamorphosis in frogs. PCBs are reported to interfere with retinoic acid activity (Burkhart et al., 1998), and may exert effects *via* this mechanism (Vandersea et al., 1998).

In humans, age at which exposure occurs (i.e., during certain developmental stages) is also critical to assessing potential effects of EDCs (see Chapter 5). *In utero*, neonatal, and exposure during childhood and puberty appear to be critical developmental periods potentially susceptible to interference by EDCs. For example, exposure to EDCs during brain development can have permanent effects, whereas similar exposures to a fully differentiated brain could have no detectable effects. It has been demonstrated that the effects of exposure to PCBs on neurobehavioral function in children will vary depending upon whether exposure occurs prenatally or postnatally (Jacobson and Jacobson, 1999). Both the severity and type of effect observed are affected by the dose and duration of PCB exposure. Recently, Longnecker et al., 2001, have shown that *in utero* exposure to DDT is associated with small-for-gestational age babies at birth; whereas, postnatal exposure has no detectable effects on child growth. Exposure data must be collected during critical periods of development in order to adequately assess the potential impacts of EDCs in both wildlife and humans. This is a difficult but crucial task, and has broad implications for designing future EDC monitoring and exposure assessment studies.

Some data on the magnitude and temporal trends of global exposure to POPs is available, but often the data were collected, analyzed, or reported in different formats making it difficult to compare data sets and evaluate new data. In general, environmental and tissue levels of certain chlorinated POPs (e.g., PCBs) have declined in some countries in response to regulations banning

or phasing out these chemicals (UNEP, 2000), but they remain of concern in many countries; and uncertainty still exists regarding future trends. In contrast to the chlorinated compounds, the levels of certain polybrominated compounds (e.g., PBDEs) appear to be increasing (Meironyté et al., 1999). As part of the global treaty on POPs, UNEP is initiating additional regional monitoring programs for potential EDCs (UNEP, 2000).

Most of the data on EDC exposure involves relatively highly exposed adult populations. Very little data is available on lower levels of exposures at different life stages, although some countries are starting to initiate exposure-monitoring programs in children and pregnant women (Needham and Sexton, 2000; Nóren and Meironyté, 2000). Very little information is available for the less persistent EDCs (e.g., phthalates, alkylphenols) with the exception of tributyltin where impacts and exposures have been evaluated in more detail. Thus, the questions remain. Where, when, how, and for whom exposures to EDCs are likely to occur in various regions of the world? What are the similarities in exposures between developing and developed countries for both humans and wildlife? What populations and groups are most vulnerable? In order to adequately address these questions, a global, coordinated, collaborative monitoring and research program on EDC exposure/response relationships is urgently needed.

## **6.2 General exposure issues**

As mentioned previously, exposure issues and methodologies for EDCs are generally similar to those of other chemicals. This section briefly summarizes the following general exposure issues: sources of exposure, fate and transport in the environment, exposure pathways in various media, bioavailability, bioaccumulation and pharmacokinetics, and internal dose (see Figure 6.1).

**[insert Figure 6.1 here]**

### 6.2.1 Sources

The wide range of chemicals shown to possess hormonal activity has been noted previously. Some environmental EDCs may be released into the environment intentionally (e.g., pesticides), but for most environmental contaminants release is unintentional. Unintentional release of chemicals can occur throughout part or all of the chemical's lifecycle (e.g., manufacturing, use, disposal). "Dioxin-like" chemicals (e.g., PCDDs/PCDFs) are formed unintentionally as by-products in a variety of industrial and combustion processes (Fara, 1999, Tobin, 1986). Leakage from landfill areas and distribution *via* sewage sludge are also sources of exposure (Daughton et al., 1999). Exposure to naturally occurring EDCs such as the phyto- and fungal-estrogens, which are important components of some human and wildlife diets, occurs globally. The isoflavonoid phytoestrogens are found in soy and legumes, the lignanes in grains and many fruits and vegetables, and the coumestans in clover and alfalfa. All have relatively short half-lives in humans and metabolites can be detected in urine and faeces. Phytoestrogens have also been detected in effluents from pulp mills resulting in reproductive effects in certain fish species (See Chapter 4). The extent of exposure to natural EDCs will vary dramatically among species, individuals, and localities.

### 6.2.2 Exposure pathways

The range of different physicochemical characteristics possessed by EDCs means that these chemicals would degrade and behave in different ways in the environment impacting on exposure routes for both humans and wildlife. The main abiotic factors that enhance the degradation processes are elevated temperature, increased sunlight, and aerobic conditions so degradation rates might be expected to be faster in warmer, sunnier parts of the world. Other processes (e.g., hydrolysis, oxidation, radical and photochemical reactions) may also transform chemicals in the environment. In contrast, some suspected EDCs (e.g., POPs) do not readily degrade in the environment and may accumulate in some compartments (e.g., in sediment), or be transported long distances from their original sources.

Exposure can occur *via* air, water, soil, sediment, and food and consumer products. The chemical may then enter the organism by ingestion, inhalation, skin contact (including *via* the gills) across cell membranes and then be absorbed into the bloodstream (Crosby, 1998). The contribution of various exposure pathways will vary among species and life-stages; and are briefly summarized below.

#### 6.2.2.1 Air

The air concentration of any chemical is dependent on its inherent and relative volatility when in contact with water, vegetation and soils. Meteorological conditions (e.g., temperature, wind speed, humidity) will influence the air concentration and should be considered if assessing exposure regionally or globally. Less volatile chemicals may also be present in particulate matter in air. Semivolatile substances, which include the majority of known EDCs, may be more or less strongly bound to particulate matter in the air thus affecting possible absorption into the bloodstream, and uptake *via* the gastrointestinal tract. Other EDCs in air may be deposited in terrestrial systems on leaves, needles, grass, soil (Jones et al., 1994) and in aquatic systems where they may enter the food chain (Stapleton et al., 2001). The air concentrations of EDCs and their distribution between particulate matter and the gas phase influence the capacity for long-range transport and thus indirectly for exposure *via* marine, limnic and terrestrial systems. EDCs of potential importance for exposure *via* air consist of those with low masses, such as persistent halogenated compounds (e.g., lindane) and non-halogenated mono-, bi- or polycyclic aromatic hydrocarbons, phenols, and phthalate esters. Calculation of the absorbed dose of an EDC from air requires experimental data or estimates from models. It is possible to measure, or calculate, the inhaled dose in humans if the air concentrations, breathing rate, and absorption efficiency are known (Paustenbach, 2000). Although a number of potential EDCs are regularly monitored in air as part of national air monitoring programs, such data is rarely collected to specifically assess exposure to EDCs in wildlife or humans.

#### 6.2.2.2 Water

Water is both the surrounding medium of a large number of water-breathing species (e.g., fish, aquatic invertebrates) and it is consumed by humans and terrestrial species. A variety of

pesticides, industrial chemicals, and natural hormones have been detected in surface waters. Chemicals may be dissolved in water and/or bound to particulate matter. In water-dwelling species, uptake can occur through direct contact *via* the gills or as they feed. Bioconcentration of semi-persistent and persistent organic chemicals depend on the equilibrium partitioning between body lipids and the ambient water, with the gill surface playing an important role (MacKay and Fraser, 2000). Fish and/or mussels have been shown to accumulate nonylphenols (Larsson et al., 1999, Lye et al., 1999, Wahlberg et al., 1990); halogenated phenols (Asplund et al., 1999); and ethinyl-estradiol (Larsson et al., 1999) from surrounding waters.

Drinking water is a potential source for human exposure to EDCs, although it is not a major exposure pathway unless unusual contamination has occurred. In developed countries drinking water is generally treated to remove microbial contamination, suspended particulate matter and some hazardous chemicals (e.g., pesticides, aromatic hydrocarbons). In some instances, other chemical contaminants are introduced as part of the water treatment process (IPCS, 1999). In developing countries, drinking water is not generally treated, and it is often contaminated with industrial and naturally occurring chemicals. Models are available to calculate the absorbed dose of a contaminant from water (IPCS, 1999). Drinking water is not regarded as a major source for exposure to persistent lipophilic chemicals (de Witt et al., 2000; Liem et al., 2000).

#### 6.2.2.3 Soil

A number of potential EDCs (e.g., PCBs, dioxins, PBDEs) have been detected in soils and/or sewage sludge in different parts of the world (Lega et al., 1997; Hale 2001; Kocan et al., 2001; Stevens et al., 2001). For certain wildlife species (e.g., worms, snails, insects) which live in close contact with the soil, this may be a major route of exposure. These organisms are a part of the food chain for certain birds and terrestrial animals. Farm animals may be exposed to contaminated soil through grazing and thus contribute to human exposure *via* this food chain pathway.

#### 6.2.2.4 Sediment



Sediment may be a pathway of exposure for certain wildlife species living in close contact with or in sediments for all or parts of their lifecycle. Some chemicals will partition to particulate matter suspended in water which may be deposited and accumulated in sediments. The continuous fallout and redeposition of atmospheric particulate matter to which lipophilic substances may be bound also increases exposure *via* this pathway. Data on PCDDs/PCDFs, PCBs and some brominated flame-retardants in sediments from European estuaries are available (Sellström et al., 1999a, van Zeil, 1997; Olsson et al., 2000). Less persistent chemicals such as alkyl ethoxylates and nonylphenols have also been reported in sediments (Bennett et al., 1998, Lye et al., 1999, Kipariss et al, 2001) as have estrogenic and androgenic steroids (Thomas et al., 2001). Human exposure *via* this route is low and restricted to consumption of bottom-feeding organisms.

#### 6.2.2.5 Food

Ingestion of EDCs and potential EDCs via food intake is generally considered the major exposure route both for humans and most wildlife and may lead to bioaccumulation and biomagnification. The contribution of dietary exposure will vary as a function of dietary preferences, position in the food chain, species and quantities consumed. Persistent, lipophilic organic pollutants often bioaccumulate in species at the top levels of the food chain. Fish-eating birds and marine mammals have been found to have concentrations of POPs many times higher than those found in fish on which they feed; or compared to levels in the surrounding waters. Sometimes the levels can be elevated by a factor of hundreds or millions (SEPA, 2001). For example, in Baltic grey seals total DDT and PCB concentrations in fatty tissues averaged 100 times higher compared to their major food source; herring (Bignert et al., 1998c). Polar bears, which constitute the end of the marine food webs of the Arctic have total PCB levels 100 times higher than levels in Scandinavian women (Henriksen et al., 2001). The high PCB levels in these top predators are due to bioaccumulation of very specific PCB congeners.

In general, marine mammals, predatory and piscivorous birds and predatory fish have higher concentrations of POPs than terrestrial wildlife and wildlife at lower trophic levels (Jansson et al., 1993). It is among these animals that effects such as reproductive failure, teratogenic effects, and eggshell thinning have been observed (see Chapter 4). In certain wildlife species, pups exposed to EDCs in the fat of mother's milk may consume much higher levels of contaminants per kilogram body weight during the lactation period than during later life stages.

Humans tend to consume both plant and animal food; and since food choices vary among population cultures and geographic regions, exposure to potential EDCs will differ considerably from one person to another. Humans relying on contaminated species for subsistence foods have been shown to accumulate higher levels of POPs (Borrell et al., 1993; Hansen et al., 1998; Lindström et al. 1999). Some of the highest concentrations of POPs have been observed in populations such as the native Inuits in northern Canada and Greenland (Ayotte et al., 1997; Hansen et al., 1998) and women from the Faroe Islands (Fängström et al., 2002). Similarly, heavy consumers of fatty fish from contaminated have been shown to have higher residues of persistent lipophilic chemicals (Jacobson et al., 1996; Svensson et al., 1991; Svensson et al., 1995). For infants, breast milk may be a major source of exposure to EDCs, coinciding with a sensitive time for growth and development. Very little is known about EDC levels in bottled baby milk or infant foods.

Human exposure to chemicals *via* food can be assessed directly through chemical analysis of food items in duplicate diet studies, or indirectly through market basket/total diet surveys (e.g., food diaries, food frequency questionnaires). Total diet surveys have been carried out for many years in a number of countries to ensure the safety of national food supplies (FAO/WHO, 1995). Methods for estimating exposure to EDCs *via* food must be adapted to specific situations since diets vary greatly in different countries and different subpopulations.

Human dietary exposure to persistent lipophilic chemicals has been relatively well studied. An extensive assessment of the dietary intake of PCDDs/PCDFs and dioxin-like PCBs in ten countries was recently published by the EU (SCOOP, 2000). Other studies (Darnerud et al., 2000) indicate that in developed countries dairy products, meat, and fish and fish products are the most important sources of exposure with proportions dependent on the source of the food item (e.g. fish from the Baltic Sea) or the exposure situation (e.g. cows milk from the Netherlands) (Liem et al., 2000).

#### 6.2.2.6 Consumer products

For humans, a wide range of consumer products (e.g., cleaning products, personal products, cosmetics, garden chemicals) provides a pathway of exposure *via* inhalation,

ingestion, and dermal contact. Of particular concern is the potential for phthalate ester exposure in young children orally by chewing on toys and teething rings (Steiner et al., 1999).

### **6.2.3 Intake and uptake**

Intake is associated with inhalation and ingestion routes of exposure while uptake is associated with dermal contact. If there is no active transport across cell membranes, absorption is dependent on the concentration gradient and ability of the chemical to cross cell membranes. Chemicals with molecular masses up to almost 1000 daltons have been shown to be bioavailable and to be transferred over biological membranes (El Dareer et al., 1987). Most environmental potential EDCs have masses in the range of 200-600 daltons. If bioavailable, the internal dose is similar to the concentration in the medium (usually blood) in the vicinity of the site for absorption or uptake. The internal dose may be much higher locally due to specific binding properties of the EDCs or their metabolites in certain cell types or tissues, since some EDCs may bind to hormone transport proteins or cellular receptors (Lans et al., 1993; Lund et al., 1988; Brouwer et al., 1998; Poellinger, 2000). Active transport mechanisms may also play a role in the absorption of EDCs (Tsuji and Tomai, 1996).

### **6.2.4 Internal Dose and Pharmacokinetics**

The internal dose is the amount of the chemical absorbed and able to undergo metabolism, transport, storage or elimination. Principal matrices for determination of the internal dose are blood, breast milk, adipose tissue/blubber and muscle tissue. It is possible to obtain all these from wildlife but they require invasive sampling except for milk, which presents particular practical problems with sampling. For humans, blood and mothers milk are relatively easy to sample, but ethical and social considerations must be taken into account. Internal exposure data are more difficult to obtain for substances with short half-lives unless they are present at steady-state levels in the tissue. For readily metabolised compounds with characterized metabolites, exposure may be estimated from the concentration of metabolites in excretion products. The nature of the exposure and the time course of metabolism, transport, storage and elimination of a chemical have important implications for when to collect samples. Measurements of the internal dose have a number of advantages including demonstration that exposure has occurred,

integration over all exposure routes and inclusion of dosing from internal sources (e.g., remobilization of lipid-soluble EDCs). Limitations include lack of information about sources and routes of exposure, the timing issues mentioned above and the general lack of background information for comparison.

The pharmacokinetics of chemicals plays a major role in determining exposure (van Birgelen and Van den Berg, 2000). EDCs can exert hormonal activities either due to their intrinsic activity or through their metabolic products (see Chapters 4 and 5). Persistent lipophilic chemicals, such as PCBs, may form both persistent metabolites and less persistent phenolic type metabolites (Letcher et al., 2000). Several aromatic compounds are known to form hormonally active metabolites, such as DDE from DDT (Metcalf, 1973); methylsulfonyl-DDE from DDE (Lund et al., 1998); and polychlorobiphenyls (OH-PCBs) from PCBs (Letcher et al., 2000, Sundström et al., 1976). A number of PBDEs can be biotransformed to OH-PBDEs (Örn and Klasson Wehler, 1998); and alkenylphenol ethoxylates to alkylphenols (Sharpe et al., 1995; Nimrod and Benson, 1996). EDCs and their metabolites are transported within organisms by the same routes and mechanisms as all xenobiotics. The uptake of chemicals through the blood/brain barrier across the placenta is of great importance, and is influenced by the structure and polarity of the chemical. For example, it appears that phenolic compounds are more easily transferred to the foetus than neutral compounds (Sauer, 2000; Guvenius, 2002). Examples of specific retention of certain EDCs in tissues other than lipid include the liver (PCDD/PCDF and other dioxin-like chemicals) (Birnbaum and Tuomisto, 2000); lung (PCBs, methylsulfoxes) (Brandt et al., 1985, Lund et al., 1985); blood retention of phenolic substances (Bergman et al., 1994, Sandau et al., 2000, Sjödin et al., 2000a) and binding of a DDE methyl sulfone to the adrenal cortex (Lund et al., 1988).

### **6.3 Case studies**

In this section, several case studies are summarized for both wildlife and humans to illustrate the range and types of exposure information available for selected potential EDCs in different parts of the world. Only general conclusions are discussed; the data supporting these conclusions is compiled in Annex I.

#### **6.3.1 Wildlife exposures**

The following case studies were chosen to illustrate the diversity of routes of exposures and species affected, and the importance of biomagnification of persistent EDCs through the food chain. Exposure information is summarized for concentrations in the environment and in biota, and where available, on temporal trends.

#### 6.3.1.1 *Persistent, lipophilic EDCs*

As mentioned earlier, the most comprehensive data sets available focus on certain POPs such as DDT and PCBs. Selected exposure data is summarized here from 3 diverse regions of the world representing different ecosystems: 1) the Baltic Sea, a marine enclosure; 2) the Great Lakes in the USA, a freshwater lake system; and 3) the Arctic, a relatively harsh remote environment. Table 6.1 lists examples of persistent EDCs, which have impacted these regions, and species which have been affected and/or studied.

[insert Table 6.1 here]

##### 6.3.1.1.1 *The Baltic Sea*

*Environment:* The Baltic Sea is one of the marine areas that has been most seriously polluted by DDT and PCBs, due to the presence of highly industrialised communities within its drainage areas. This ecosystem is located in the temperate zone of the world with clear seasonal variations. In the north, the surface is generally covered by ice for some months whereas the southern areas are rarely ice-covered. The water is brackish, the tidal movement is a few centimetres in the south and imperceptible in the north. Since the Baltic Sea is a marine enclosure, the importance of dilution processes by exchange of clean water from the North Atlantic is small. Furthermore, the water circulation within the Baltic, is reduced because of a strong halocline, with higher salinity in bottom water and lower salinity in surface water. The surface water temperature has higher seasonal variations than most other marine waters. There are comparatively few species in the Baltic Sea. The biological community comprises a mixture of marine species (herring, cod, seals, etc) as well as freshwater species (perch, pike etc). For the top consumers, the fatty herring is an important food item and its role in bioaccumulation to marine birds and mammals (as well as to man) explains some of the detrimental effects found in the Baltic Sea ecosystem.

Potential endocrine-mediated adverse effects have been observed in a number of species in the Baltic Sea. Examples include an increased prevalence of female salmon producing offspring with a low survival rate (Johansson et al., 1995); lowered reproductive capacity and eggshell thinning in white-tailed sea eagles (Stjernberg et al., 1990; Helander et al., 1998; Helander et al., 1999b; Odsjö et al., 1977); razor bill (Andersson et al., 1974) and guillemot (Bignert et al., 1995); and immune and reproductive impairment in marine mammals (Bergman et al., 1985; Simms and Ross, 2001).

*Concentration data:* The concentrations of various EDC compounds in some selected matrices in representative Baltic species are summarized in Annex I (Tables 1-10). Extensive data are provided for herring and salmon, since both are important food items for consumers at the top of the food chain including man. These summary tables illustrate the difficulties encountered when trying to compare different studies. In some instances, sampling was carried out at different times of the year, organisms were sampled at different stages in the lifecycle, samples were collected and analyzed using different methods, and results were analyzed and reported using different formats. In order to make the data comparable to some degree, data are provided in these tables on a lipid weight basis in  $\mu\text{g/g}$ , which required estimation of fat content in some matrices and species. Concentrations of DDT and PCBs were highest in white-tailed sea eagle eggs sampled during the 1960's and 70's and although these levels decreased during the 1980s and 90's, concentrations remain high compared to species lower in the food web. Figure 6.2 and 6.3 summarizes data on total PCB and DDT concentrations for species at several trophic levels for the periods 1969-72 and 1988-98.

[insert **Figure 6.2** and **6.3** here]

*Temporal trends:* Monitoring studies of the Baltic environment have covered a number of species, including terrestrial, freshwater and marine biota (Bignert et al., 1998a). Interestingly, annual changes over time have been shown to be very similar for the compounds measured, whether the organisms were from terrestrial, freshwater or marine environments (Bignert et al., 1998b, Bignert et al., 1998c, Olsson et al., 1997, Olsson et al., 1998). However, no significant decrease of dioxins was observed in guillemot eggs and herring from the Baltic over the last 10 years, while DDE and HCH levels continued to decrease (Olsson et al., 2002).

Data on temporal trends is summarized in Annex I (Figures 1-7). Total PCB concentrations of guillemot eggs declined from around 300  $\mu$  g/g lipid in 1969 to around 30  $\mu$  g/g lipid in 1999. Similar trends are shown for DDTs, HCBs and TEQs of PCDDs/PCDFs in guillemot eggs (see Annex I). Except for PBDEs and chlordanes, concentrations have decreased since the beginning of the 1970s (Bignert *et al.*, 1998a, de Wit *et al.*, 1994, Odsjö *et al.*, 1997). The temporal trends for  $\alpha$ -HCH and  $\gamma$ -HCH (lindane) as determined in herring showed similar decreases. Decreasing concentrations of toxaphene during 1974-1989 in the environment have also been reported (Wideqvist *et al.*, 1993). For PBDEs, temporal trends show a different pattern. Concentrations of PBDEs in guillemot eggs increased from the late 1970s to the late 1980s and then declined (Kierkegaard *et al.*, 1999, Moilanen *et al.*, 1982, Sellström *et al.*, 1999b).

The decreasing concentrations of OCs mentioned above have been followed by a concurrent improvement in reproductive capability for white tailed sea eagle (Helander *et al.*, 1999a), in eggshell thickness in guillemot (Bignert *et al.*, 1995) and in increasing populations of otter (Roos *et al.*, 2000), mink (Alsberg *et al.*, 1993), gray seal (Helander *et al.*, 1999b), harbor seal (Helander *et al.*, 1992) and ringed seal (Härkönen *et al.*, 1999). Although reproductive health has improved in seals, some problems remain among gray seal (Bergman, 1999) and ringed seal (Mattson *et al.*, 1995) (See Chapter 4).

#### 6.3.1.1.2 The Great Lakes

*Environment:* The Great Lakes comprise a large system of connected freshwater lakes lying mainly along the US - Canada border. Water flow is from Lake Superior (with fairly oligotrophic water) in the north-west through Lake Michigan (with heavy industries) just south of Lake Superior, via Lake Huron, Lake Erie and Lake Ontario (the latter three lakes are highly eutrophic and polluted by both municipal, agricultural and industrial effluents) to the St. Lawrence River. Numerous large cities are located along the shores of these lakes, many with heavy industries including producers of pesticides and organochlorine compounds. This has led to widespread contamination by such substances from air, the watersheds and from industries and urban areas located along the shoreline. Lake Superior is considered to be less contaminated than the other lakes and less influenced by local industries. Most of the lakes have been highly contaminated with PCBs, DDT, PCDDs/PCDFs (particularly 2,3,7,8-TCDD) and a number of organochlorine pesticides.



Potential endocrine-mediated adverse effects have been observed among species in the Great Lakes area (see Chapter 4). These include poor reproduction in fish, such as Chinook salmon (*Oncorhynchus tshawytscha*) and lake trout (*Salvelinus namaycush*), and birds including herring gulls (*Larus argentatus*) (Fox et al., 1998), Forster's terns (*Sterna forsteri*), double-crested cormorants, Caspian terns (*Sterna caspia*) (Gilbertson, 1989)) and bald eagles (*Haliaeetus leucocephalus*), (Best et al., 1994).

*Concentration data:* Concentrations of DDT, PCBs and PCDDs/PCDFs in representative species for the Great Lakes are summarized in Annex I (Tables 11-13; Figures 8-9). Data are taken from studies carried out in the 1980s-1990s where sufficient quality control/quality assurance could be obtained. PCB levels are reported as total PCB or the sum of varying numbers of PCB congeners. TCDD toxic equivalents (TEQs) were calculated from reported concentrations of PCDDs/PCDFs. Similar to the Baltic Sea observations, concentrations of DDT/DDE, PCBs and PCDD/PCDFs were highest in herring gull bird eggs sampled in the early 1970's. Although levels decreased during the 1980s and 1990s, the concentrations remain higher in herring gulls reflecting their position at the top of the food web.

*Temporal trends:* The Canadian Wildlife Service has monitored herring gull eggs annually from 16 sites covering all 5 Great Lakes for levels of DDT, PCBs and dioxins (see Annex I; Figures 10-13). An analysis of the temporal trends for PCBs and DDE from 1974 to 1995 indicates that concentrations of these have generally declined in all the Great Lakes (Pekarik et al., 1998). However, temporal trends vary among individual sites. For example, in Lake Michigan, Huron, Erie and Ontario, PCB levels have continued to decline at the same rate from 1974-1975, whereas the rate of decline for PCB concentrations in Western Lake Ontario slowed between 1987-1995, and ceased after the mid-1980s in Lake Superior. For Lake Michigan (Green Bay area), PCB concentrations have shown no significant temporal trend since 1976. Organochlorine concentrations have also declined significantly in double-crested cormorant eggs between 1970 and 1995 in Lakes Ontario, Superior and Huron, but not in Lake Erie (Ryckman et al., 1998). Lake trout from Lake Michigan, Ontario, Huron and Superior have been monitored for PCB and DDT concentrations from the 1970s to the 1990s. For Lake Ontario, data also exists for dioxin-like compounds. Concentrations for all these compounds have generally declined from the 1970s to the 1990s (Borgmann et al., 1991, De Vault et al., 1986, Huestis et al., 1996, Huestis et al., 1997). In contrast, PBDE levels have continuously increased 20-60 times from 1981 to 1999 in herring gull eggs from Lake Huron (Channel/Shelter Island), Lake Michigan (Gull Island) and



Lake Ontario (Snake Island), respectively (Wakeford, 2000). A continuous increase in PBDE concentrations has also been observed in Lake Ontario Lake trout caught during 1978-1998 (see Annex I; Figure 13) (Luross et al., 2000). Low concentrations of PBDEs have been reported in Lake Michigan Lake trout (Manchester-Neesvig et al., 2001).

#### 6.3.1.1.3 *The Arctic Region*

*Environment:* The Arctic region has a unique cold climate with extreme seasonality in light and productivity. Lakes and large sea areas are covered by ice much of the year. Species diversity is low and food webs are relatively simple, but include third level carnivores (polar bears). Primary production is higher in aquatic ecosystems than in terrestrial ecosystems. The ecological systems are generally oligotrophic. Lipids play an important role as an energy source in the Arctic food web, which leads to high bioaccumulation and biomagnification of lipophilic endocrine disruptors in upper trophic level animals. The extreme seasonality of the Arctic also leads to large fluctuations in the lipid stores of many organisms because of the need to store fat during the short productive season and consumption of stored fat when food is scarce. This in turn can lead to large fluctuations in blood concentrations of EDCs with consequent redistribution in tissues, making it difficult to estimate dose-response relationships in some species.

Since 1981, the Arctic Monitoring and Assessment Programme (AMAP) has monitored levels of certain environmental pollutants (de March et al., 1998). Concentrations of DDT, PCB, HCH, HCBs and PCDD/PCDFs are summarized in Annex I (Tables 14-15; Figures 14-18) for representative species in the Arctic. These data extracted from AMAP studies (de March et al., 1998) reflect the highest and lowest concentrations of these chemicals measured anywhere in the Arctic. PCB levels are given as total PCB or the sum of varying numbers of congeners. TEQs are based on reported values. Data represent studies carried out in the 1990s where sufficient quality control/quality assurance could be obtained. Several studies indicate higher concentrations of PCB and DDT in certain parts of the Arctic (e.g., Russia), but the database is incomplete and further monitoring data is needed.

Concentrations of POPs in Arctic species reflect their position in the food web. For example, eggs of top prey species such as white tailed sea eagles had higher concentrations than species lower in the food web. Studies of Canadian seabirds have shown that eiders, which over winter in contaminated waters, have a higher contaminant load than birds over wintering in clean

waters. In mammals, concentrations in marine species are higher than in terrestrial species and highest in top predators at the end of long food chains (e.g., polar bears) (de March et al., 1998). Higher OH-PCB concentrations than PCB concentrations were found in the blood of polar bears (Sandau, 2000).

*Temporal trends:* Monitoring in Arctic biota from Canada, Greenland, Norway and Finland has been limited to small and infrequently collected samples (2-4 times over 25 years), making it difficult to determine temporal trends. Furthermore, high intrasite variability and changes in analytical methodology have made temporal comparison problematic. Generally speaking, limited temporal trend data indicate declining concentrations of PCB and DDT in the Arctic. Less is known about the temporal trends of many other persistent compounds, including HCHs, HCB, chlordane, toxaphene, dieldrin, and PCDDs/PCDFs.

Annual collection and analyses in pike in Lakes Storsjön (Annex I; Figure 17) and arctic char in Lake Abiskojaure (Annex I; Figure 18) in sub-Arctic Sweden for the past 20-30 years have provided some of the strongest evidence for declining levels of DDTs and PCBs northern Scandinavia (Bignert et al., 1998a). A sudden decline occurred soon after measures to reduce the discharges of DDT and PCB were implemented in the early and middle 1970s (Bignert et al., 1995, Bignert et al., 1998a, Olsson et al., 1986). Since that time, the annual decline of DDT and PCB concentrations (3-8% a year) has continued (Bignert et al., 1998a).

#### 6.3.1.1.4 *Global distribution of DDT and PCB compounds in marine mammals*

Marine mammals (e.g., harbor seals) represent species at the top of the food chain; and considerable monitoring data has been collected on tissue levels of POPs from various marine mammals in different parts of the world (see Annex I; Table 16). Data on polar bears is also included since they prey on seals in the Arctic. Figure 6.4 illustrates the range of PCB and DDT levels in fish-eating marine mammals from various parts of the world. Examples of effects of PCB and DDT contamination in marine mammals includes: low reproductive success and declining populations of harbor seals in the Dutch Wadden Sea (Reijnders, 1980; Reijnders, 1986; Reijnders, 1990); premature pupping in California sea lions (Gilmartin et al., 1976), and immune and reproductive impairment in Baltic ringed and gray seals (Bergman and Olsson, 1985; Roos et al., 1998).

Factors that affect tissue concentrations include species differences, food, age and sex differences, seasonal differences, and differences in the chemical analytical methods. Overall, the available data gathered indicates worldwide occurrence of POPs, including in the remote areas of the world. These data are scattered and spread over a large number of species, collection year, habitat, seasons, and chemical analysis methodologies making it difficult to compare data and assess temporal and geographical trends.

[insert Figure 6.4 here]

#### 6.3.1.2 Tributyl tin

Tributyl tin (TBT) is found in antifouling paints applied to hulls of ships. TBT degrades in the environment to the less active di- and mono-butyl tins. Studies on TBT concentrations in the environment and in biota have been carried out many locations throughout the world including coastal areas around Europe, Asia, North America and Australia as well as open ocean areas (Guruge et al., 1996, Guruge et al., 1997, Iwata et al., 1995, Kannan et al., 1995a, 1995b, 1996, 1997a, Kannan and Falandysz, 1997, Kim et al., 1996a, 1996b, 1996c, Takahashi et al., 1997, 1999, Tanabe et al., 1998). Coastal areas are impacted by previous and current use of TBT on boats and fishing equipment near harbours and marinas. Sediments in particular seem to be reservoirs for TBT even after use has stopped, leading to continued exposure. Open ocean areas are exposed to TBT from large vessels that continue to use TBT on the ship's hulls. TBT has been reported to cause imposex (induction of a penis in females) in marine gastropods at very low concentrations and shell abnormalities in some species of bivalve molluscs (see Chapter 4).

*Concentrations in the environment:* Concentrations of TBT have been determined in water column and sediment samples from freshwater, estuaries, seawater, marinas and harbors (see Annex I).

*Concentrations in biota:* Species analyzed for TBT include invertebrates, fish, eels, birds and marine mammals. Analysis of TBT levels in fish and mammals has only been carried out since the mid-1990s. Higher concentrations are found in comparable matrices collected near coasts compared to those collected from the open ocean. For the Pacific Ocean area, highest concentrations are found in fish around Japan and Australia, with lower levels around developing nations such as India, Bangladesh, Thailand, Indonesia and Vietnam. Concentrations in fish from

the U.S. coast, Italy and the Baltic Sea are fairly similar to each other and to concentrations in Japan.

Concentrations of TBT in various species are highly variable, due to differences in external exposure and metabolism (Annex I - Table 17). This is exemplified by monitoring studies of Otsuchi Bay on the Pacific Coast of northern Japan (Tanabe, 1998; Takahashi et al., 1999). Levels are generally low in invertebrates and fish compared to marine mammals. Cormorants from Lake Biwa, Japan had total mono-, di- and tributyltin concentrations of 140-1000 ng/g wet weight in liver (Guruge et al., 1996). Diverse species of seabirds from around the Japanese and Korean coast had TBT concentrations ranging from non-detectable to 500 ng/g wet weight in liver, whereas oceanic birds had concentrations ranging from nondetectable to 29 ng/g wet weight in kidney (Guruge et al., 1997). Along the Polish coast of the Baltic Sea, several species of seabirds had TBT concentrations of 35 - 4600 ng/g wet weight in liver (Kannan et al., 1997a).

In marine mammals (primarily cetaceans and pinnipeds), a similar geographic trend is seen as for fish. Liver tissue samples from various species of cetaceans from the Japanese coast had 230 - 10 200 ng/g wet weight, from China, 350 - 1200 ng/g wet weight and from the Philippines and India 40 - 200 ng/g wet weight (Tanabe et al., 1998). Liver tissue samples from several cetaceans from the U.S. Atlantic coast had 80 - 11 300 ng/g wet weight, (Kannan et al., 1997). Bottlenose dolphins from the Italian coast had 1200 - 2200 ng/g wet weight and neonatal harbour porpoise from the Baltic Sea had 18 - 27 ng/g wet weight in liver (Kannan and Falandysz, 1997). Liver tissue sample from pinnipeds from Japan had TBT concentrations of 50 - 320 ng/g wet weight (Tanabe et al., 1998) whereas along the Alaskan coast, concentrations were 2 - 24 ng/g wet weight in liver (Kim et al., 1996a).

*Temporal trends:* Because of its high toxicity to marine invertebrates at low water concentrations, many countries restricted TBT use during the 1980s. In France, restrictions were imposed in 1982 and over the period 1982-1985, water and oyster concentrations of TBT dropped 5-10 times, resulting in decreased oyster shell malformations and increased populations (Alzieu, 1991). Use restrictions have also led to reductions in concentrations in various media (water, sediment) and organisms (oysters, mussels, dog whelk) from Japan, US and the UK, with concomitant increases in oyster shell growth and decreased imposex in dog whelk (Harino et al., 1999, Miller et al., 1999, Valkirs et al., 1991, Waite et al., 1991). Where exposure to TBT had

caused irreversible imposex in dog whelks there was a time lag in population recovery (Miller et al., 1999). No temporal trend data are available for fish, birds or marine mammals. In Germany, restriction of TBT in 1989 resulted in decreasing TBT concentrations in freshwater fish from several German rivers. However, mussel and eelpout from the Wadden Sea (The Netherlands) showed no obvious decline in TBT concentrations over the period 1985-1998 (Umweltbundesamt, 2000).

#### *6.3.1.3 Alkylphenols and ethoxylates and their degradation products (APEs)*

##### *Sources and Fate of APEs*

Alkylphenol polyethoxylates (APEs) are high-volume chemicals that have been used for more than 40 years as detergents, emulsifiers, wetting agents and dispersing agents. APE-containing products are used in many sectors, including textile processing, pulp and paper processing, paints, resins and protective coatings, oil and gas recovery, steel manufacturing, pest control products and power generation. Certain APEs are also used in a wide range of consumer products, including cosmetics, cleaners and paints, and in a variety of applications. It is therefore not surprising that they have been widely detected in effluents and the environment.

APEs with more than eight ethoxy (EO) units (most common commercial products) are readily degraded in effluent treatment system with >92% efficiency (Ahel et al. 1994a,b; Kubek and Naylor 1990; Naylor 1995; Brunner et al. 1988; Giger et al. 1987). The primary products remaining in effluents and sludge after treatment are APEs with low EO chain lengths, alkylphenol polyethoxylates (AP<sub>1,2</sub>EO), alkylphenol polyethoxycarboxylates (AP<sub>1,2</sub>EC), as well as alkylphenols (AP), which may ultimately be mineralized to CO<sub>2</sub> (Ahel et al. 1994; Ball et al. 1989; Yoshimura 1986; Giger et al. 1984; Lee and Peart 1995). APEs therefore occur as a complex mixture in final effluents of municipal or industrial treatment systems (Lee et al. 1999; Bennie 1999). The range of concentrations as well as the relative proportions, are dependent on the sources, as well as degree and type of treatment (Maguire 1999; Ahel et al. 1994). As the EO chain length decreases, a corresponding decrease in water solubility is observed. The APs and low chain length APEOs are therefore generally associated with organic particles, and sludges in the treatment system. In contrast, nonylphenol polyethoxycarboxylates (NPECs) are considerably

more water soluble than the corresponding NPEOs and are present in the aqueous phase of final effluent (Maguire 1999; Bennie 1999; Servos et al. 2000).

### *Environmental Concentrations*

The environmental concentrations of APEs are generally low except in the vicinity of effluent discharges. However, APEs are commonly found at trace levels in surface waters and sediments around the globe. The relative distribution in water will differ from that in sediments or sludges applied to land and is dependent on the sources, treatment and environmental characteristics. The differences in the properties of the degradation products makes estimation of their environmental exposure complex, although in general, the APECs are found in the aqueous phase while the short chain APEOs and APs are associated with particles and sludge. Concentrations of APEs near industrial sites or textile industries have been reported to be relative high, e.g.  $> 1\text{--}1000\text{ }\mu\text{g/L}$  (Blackburn and Waldock 1995; Bennie 1999; Lye et al. 1999, Ahel et al. 1993). Many of these sites have recently implemented control measures or turned to alternatives and the environmental release has been reduced (Bennie 1999; Lee and Peart 1999; Naylor et al. 1992). A limited amount of data does suggest a decrease in the concentrations of these chemicals in the environment over time (Umweltbundesamt 2000). However, APEs continue to be released from a variety of sources, particularly municipal effluent treatment systems. Municipal treatment systems sampled across Canada, contained concentrations of NP that ranged from  $<0.02$  to  $62.1\text{ }\mu\text{g/L}$ , from  $0.12$  to  $4.79\text{ }\mu\text{g/L}$  and from  $<0.02$  to  $3.20\text{ }\mu\text{g/L}$  for primary, secondary and tertiary treatment systems, respectively (Bennie 1998a; Lee et al. 1998; Servos et al. 2000). In Canadian fresh water, concentrations of NP ranged from non-detectable ( $<0.02\text{ }\mu\text{g/L}$ ) to  $4.25\text{ }\mu\text{g/L}$ , mean  $0.20\text{ }\mu\text{g/L}$  with the highest concentrations being in direct proximity to municipal or industrial discharges (Bennie et al. 1997; Bennie 1998). This is consistent with similar surveys of surface waters in the USA (Naylor et al. 1992; Weeks et al. 1996) and Europe (Ahel et al. 1994; Lye et al. 1999; Blackburn et al. 1995; Larsson et al. 1999; Umweltbundesamt 2000).

Shang et al. (1999) observed the distribution of NPEs in marine sediments from the Strait of Georgia to be dominated by NP and NP1EO which were also persistent in sediments. NP concentrations in sediments from the Great Lakes basin and the upper St. Lawrence River ranged from below detection levels ( $<0.02\text{ }\mu\text{g/g}$ ) to  $72.2\text{ }\mu\text{g/g d.w.}$  (Lee and Peart 1995; Bennie et al. 1997; Bennett and Metcalfe 1998; Bennie 1998). Sediments

in rivers from the UK were reported to have similar levels from 0.03 to as high as 131  $\mu\text{g/g}$  d.w. in relative contaminated systems (Lye et al. 1997). Sediments concentrations in the River Glatt, Switzerland, were also reported in a similar range (Ahel et al. 1993; Ahel et al. 1994). The most common APE detected in sludges is NP which ranged from 0.74 to 1260  $\mu\text{g/g}$  d.w., in surveys Canadian treatment systems (Lee and Peart 1995; Lee et al. 1997; 1998; Bennie 1998; Bennie et al. 1998; Servos et al. 2000). Sludges are routinely applied to amend agricultural soils. There are few data available about the residuals of APEs in these soils, although they do appear to degrade rapidly (Bennie 1999; Marcomini et al. 1989).

There is minimal data available on APEs in biota in the published literature. Most data suggest a low to moderate potential for these chemicals to bioaccumulate (BAF 0.9-3400) but trace levels have been detected in various locations around the globe (Servos 1999). Concentrations of NP, NP1EO and NP2EO were as high as 1.6, 7.0 and 3.0 mg/kg, respectively, in four composite samples of fish (chub, barbell, rainbow trout) collected from the Glatt River in Switzerland; concentrations in a single sample of wild duck (*Anas boscas*) ranged from not detected to 1.2, 2.1 and 0.35 mg/kg d.w. for NP, NP1EO and NP2EO, respectively (Ahel et al. 1993). Eklund et al. (1990) demonstrated some limited bioaccumulation of APEs in marine organisms. Granmo et al. (1991) caged mussels (*Mytilus edulis*) near an outfall of an industrial facility producing surfactants, and reported BAFs of 340 (wet weight) for NP. Wahlberg et al. (1990) caged mussels in the effluent of a plant which produced NPEs and measured BAFs of 60 for NP3EO, 100 for NP2EO, 170 for NP1EO and 340 for NP.

### *Effects*

Several detailed reviews of the toxicity and bioavailability of APEs have been recently published by Talmage (1994), Staples et al. (1998), Nimrod and Benson (1996) and Servos (1999). Although the data in the literature are scattered among many species, different test methods and chemicals, there is a consistent pattern in the toxicity. NP is relatively toxic (LC50, EC50) to fish (17-3000  $\mu\text{g/L}$ ), invertebrates (20-3000  $\mu\text{g/L}$ ) and algae (27-2500  $\mu\text{g/L}$ ) and chronic toxicity values (NOEC) as low as 6  $\mu\text{g/L}$  in fish and 3.7  $\mu\text{g/L}$  in invertebrates have been reported. Although there are considerably less data available for the NPEOs and NPECs there is an apparent increase in the toxicity of NP and NPEs with decreasing EO chain length. NPECs



which are much more water soluble, are much less toxic than corresponding NPEOs, and have acute toxicity similar to NPEOs with 6-9 EO units. The potential of these compounds to bioaccumulate is dependent of their hydrophobicity and their varies greatly with APs having only a moderate tendency to accumulate in biological tissues.

Numerous studies have demonstrated the ability of NPEOs and their degradation products to disrupt the normal function of the endocrine systems of various organisms. Soto et al. (1991) accidentally found that *p*-nonylphenol (NP) released from polystyrene centrifuge tubes caused proliferation of human estrogen sensitive MCF-7 breast tumor cells (E-screen). They have since been reported to cause a number of estrogenic responses in a variety of aquatic organisms including: altered growth of testes (Ashfield et al. 1998), altered steroid metabolism (Tremblay et al. 1998), intersex (Metcalf and Gray 1997), histological changes (Miles-Richardson et al. 1999), and disruption of smoltification (Madsen et al 1997; Fairchild et al. 1999).

APEs, especially nonylphenol and octylphenol, bind to the estrogen receptor resulting the expression of several responses both *in vitro* and *in vivo*, including the induction of vitellogenin in fish (Jobling et al. 1996). These effects occur at a range of concentrations similar to those at which chronic effects occur in fish and invertebrates. The threshold of NP for induction of vitellogenin in rainbow trout was reported as 10 µg/L (Jobling et al. 1996), while the induction of mRNA for vitellogenin was reported at concentrations as low as 1 µg/L (Fent et al. 1999). Recent reports by Miles-Richardson *et al.* (1999) suggest that in fathead minnows, histological and biochemical effects can occur at concentrations approaching or below 1 µg/L. However, the significance of these responses is not fully understood, and the effects on the organism or population have not been determined. Recent work by Brown et al. (1998) and Madsen et al. (1997) has demonstrated that NP can affect smoltification, resulting in reduced growth and survival in Atlantic salmon (*Salmo salar*) after very short term exposures to concentrations expected in the environment. Intersex in Japanese medaka has been demonstrated at 50 µg/L (Gray and Metcalfe 1997). Although potential effects mediated through the estrogen receptor have been identified both *in vitro* and *in vivo* for NP in fish, this is only one mechanism by which a chemical such as NP can potentially interact with endocrine systems. As with acute and chronic toxicity, there are few data available on the relative potency/estrogenicity of the other metabolites, and there is considerable discrepancy among the few existing studies. Jobling and Sumpter (1993) reported that using trout hepatocytes to measure induction of vitellogenin, NP2EO and NP1EC were 0.67 and 0.63 the potency of NP.



The importance of considering APEs as a mixture rather than individual compounds has been demonstrated by Servos et al. (2001). In municipal effluents other compounds such as natural and synthetic estrogens have been identified which also bind to the estrogen receptor and cause similar biological responses as APEs (Desbrow et al. 1999; Routledge et al. 1999). In some effluents APEs may contribute only a small proportion of the total estrogenic potency although this will vary drastically depending on the sources, treatment, and compartment assayed.

#### *6.3.1.4 Summary and conclusions of wildlife exposures*

The case studies summarized in this section along with the data presented in Annex I clearly provide evidence that considerable exposure to potential EDCs (particularly POPs) has occurred in a variety of wildlife species. However, most of this exposure data comes from selected species at the top of the food chain, or from wildlife living in a contaminated habitat in Europe and North America. Exposure data for nonpersistent EDCs, other wildlife species, at low environmental levels, and in other parts of the world are generally lacking. Even with the availability of good data sets, there are difficulties in comparing exposure levels between species, over time, and in different areas because of different approaches to sampling, analytical methodologies, data reporting and statistical treatment. To adequately assess EDC-related effects in wildlife, global, long-term exposure monitoring is needed, using harmonized, consistent methodologies to ensure comparability of data. Obviously, it is impossible to measure all areas and all species at all life stages; therefore a strategic approach needs to be developed to determine what types of exposure data are needed to adequately assess the impact of EDCs on wildlife.

#### **6.3.2 Human exposures – some selected case studies**

Some case studies are summarized to illustrate human exposure to a diversity of potential EDCs. These include dioxin-like compounds (such as PCBs, OH-PCBs, PBDEs, DDTs), phthalates, atrazines, and phytoestrogens. Some information on concentrations of potential EDCs in breast milk and potential exposures of children and vulnerable populations to EDCs is also summarized.

#### 6.3.2.1 “Dioxins”

PCDDs and PCDFs, known collectively as dioxin-like compounds, are by-products of a variety of industrial and thermal processes. There are 75 and 135 congeners of PCDDs and PCDFs, respectively; however, only 7 PCDDs and 10 PCDFs are generally found in humans. Unfortunately, these include the most toxic 2,3,7,8-substituted congeners. Two chemical subclasses of polychlorinated biphenyls (PCBs), the non-*ortho*chlorinated PCBs and the mono-*ortho*chlorinated PCBs have toxicological properties similar to the PCDDs and PCDFs and are also discussed here. The ingestion of food containing trace levels of these chemicals is estimated to account for more than 90% of human background body burden.

PCDD and PCDF levels are normally reported in various matrices as concentrations of individual PCDD/F congeners or as total dioxin concentration per gram lipid in the sample calculations. Total concentrations of “dioxin-like” compounds are often calculated and reported as toxic equivalents (TEQs). TEQs are sums of concentrations weighted to account for the varying potencies of the different compounds. WHO has established a tolerable daily intake for the whole group of dioxin-like compounds of 1-4 picograms (pg) TEQs/kg body weight/day (Van Leeuwen and Younes, 2000).

*Trends:* It is clear from many reports that there has been a marked decrease over the past 20-30 years in the levels of PCDDs/PCDFs in the general population of industrialized countries (Furst *et al.*, 1992; Orban *et al.*, 1994; Pöpke, 1998; Liem *et al.*, 2000; Meironyté and Norén, 2001). Some recent data indicate this trend may not be continuing in Germany and Spain (Liem *et al.*, 2000; Furst 2001).

*Levels:* Several review articles have described levels of PCDDs, PCDFs, and the dioxin-like PCBs in the general population. Data are summarized in Annex I (Tables 19-21; Figure 19). Data sets from New Zealand (Bates *et al.*, 1999), the USA (Anderson *et al.*, 1998), Norway (Johansen *et al.*, 1996), Sweden (Norén and Meironyté, 2000), and Canada (Dewailly *et al.*, 1996). Samples collected during the 1990s from different matrices (blood, serum, breast milk) all indicate TCDD levels in the range of 2-3 pg/g lipid weight (ppt). This level is consistent with the reported level of 3.4 ppt in the fat of breast milk from 33 countries (IARC, 1997). For some PCDDs, the reported values from Europe and New Zealand tended to be lower than those from North America (Wingfors *et al.*, 1999). In contrast, 2,3,4,7,8-PCDF levels occur in higher

concentrations in samples from Europeans compared with those from North Americans and New Zealanders. The higher concentrations of 2,3,4,7,8-PCDF in the European samples largely accounts for the higher contribution to the TEQ. The TEQs of the PCDDs and PCDFs in these five data sets range from 12.6 to 24.2 ppt, which is in agreement with the range of 4-27 ppt TEQ in breast milk samples taken in 1993/94 in 18 countries (see Figure 6.6). Although levels of TEQs in industrialized countries are very consistent (Schröter-Kermani et al., 2000; Pöpke et al. (2000), there may be regions where higher levels (“hot spots”) occur. For example, higher TCDD levels have been reported in serum and breast milk from residents of Kazakhstan with levels in the range of 6.9-68.6 ppt TEQ and 1-208 ppt TEQ, respectively (Hooper et al., 1998; Hooper et al., 1999). Unfortunately, there are many parts of the world where biomonitoring has not been carried out.

[insert Figure 6.5 here]

The temporal concentration differences noted above for the PCDDs and PCDFs with the time of sample collection is not seen for the non-*orthosubstituted* PCBs. The earliest samples from the US have the lowest levels of these PCB congeners. The TEQs for the non-*orthosubstituted* PCBs from the two Scandinavian data sets are 4-5 times higher than the US samples. The reasons for this variance are not known and more complete data sets are needed to better describe total TEQ levels. Mono-ortho substituted PCBs (e.g., PCB 105, 118, and 156) can contribute greatly to the TEQ and should be included in calculations of TEQs.

Several investigators have noted increased levels of the above chemicals in humans with increasing age (Bates *et al.*, 1999). Bates et al., (1999) observed a 3-4 fold increase in serum of New Zealand individuals 65 years of age and older compared to the 15-24 year age group. Levels for males and females were similar. In contrast plasma levels of TCDD in women in Seveso, Italy, had higher levels than men; and this gender difference persisted after adjustment for location within the zone, age, body mass index, and smoking (Landi *et al.*, 1998; Pöpke, 1998).

#### 6.3.2.2 Polychlorinated Biphenyls (PCBs)

The manufacturing of PCBs has been banned in many countries since the 1970s (de March et al., 1998). However, because of their long lifetime in old electrical equipment, continued use in some parts of the world, and their persistence and bioaccumulation populations continue to be exposed to PCBs. For toxicological purposes, the PCBs have been divided into

three main classes: those containing no *ortho* substitution (non-*ortho*-PCBs), those with one chlorine in the *ortho* position (mono-*ortho*-PCBs), and those containing chlorine substitution at two or more *ortho* positions. The first group act through mechanisms similar to dioxins and may be analytically measured in the same fraction of the sample preparation; hence, they are generally reported on a lipid-adjusted basis. The mono-*ortho*-PCBs are generally not analysed in the same fraction as the PCDDs and PCDFs but have been assigned toxic equivalence factors (TEFs) (Ahlborg et al., 1994). However, this group is often reported on a whole weight (or volume) basis rather than a lipid-adjusted basis, and thus, can then not be accurately included in the total TEQ. The group containing chlorine substitution at two or more *ortho* positions is often the highest in concentrations in environmental and biological samples. Historically, they were often reported only on a whole-weight (or volume) basis (ng/g or ng/mL) in biological samples. The three congeners generally found in the highest concentrations in biological samples are: 2,2',4,4',5,5'-hexachlorobiphenyl (CB153); 2,2',3,4,4',5'-hexachlorobiphenyl (CB138); and 2,2',3,4,4',5,5'-heptachlorobiphenyl (CB180). Prior to the 1990's, most of the PCB exposure data in humans was reported as total PCBs. Since that time, exposure to PCBs has generally been reported on an individual PCB congener basis. The different ways of reporting PCB concentrations leads to major difficulties in making data comparisons. To promote future comparisons of data, measurement of congener specific concentrations should be encouraged.

*Levels in Special Populations – (Fish eaters):* Svensson *et al* (1995) showed a high correlation between consumption of fish from the Baltic Sea and PCDD, PCDF, non *ortho*-PCB and mono-*ortho*-PCB levels. Three groups were studied: individuals who did not consume any fish, moderate fish consumers (200-500 g of fish/week), and high consumers (consumption rate of 700-1750 g of fish/week). The average total TEQ in serum for these three groups was 17.5 ppt, 25.8 ppt, and 63.5 ppt, respectively. The non-*ortho*-PCB contribution to the total TEQ in the non-fish/fish consumers from Sweden was 30%. Other studies on PCB levels in populations with high seafood intake from Latvia and Sweden (Sjödin et al., 2000a); the Netherlands (Hanrahan et al., 1999) and the Great Lakes area (Anderson et al., 1998) have all reported higher total PCB levels in high fish-consuming populations compared to the concentrations in populations eating little or no fish. Recently high PCB concentrations (CB-153) were reported in a subgroup of pregnant women from the Faeroe Islands (Fängström et al., 2002). The Arctic Monitoring and Assessment Programme (AMAP), analyzed PCB levels in maternal plasma, in 1995/1996 from six of the eight circumpolar countries, and the results are summarised in Annex I (Table 21). Levels have also been measured in breast milk (Annex I - Table 21). In general, PCB

concentrations and concentration patterns were similar in all countries except Greenland, while higher concentrations were found. One group of males (consumers of Norwegian crabs) also had much higher median levels of PCBs than other AMAP participants (Jacobson *et al.*, 1996). Studies are difficult to compare due to different sampling, analytical and reporting methods, but overall the body of evidence indicates that these populations have increased exposures.

*Hydroxylated PCBs (OH-PCBs):* PCBs are transformed to hydroxylated PCBs (OH-PCBs) in both wildlife (Jansson *et al.*, 1975) and in experimental animals (Sundström *et al.*, 1976). Several reviews on the metabolism of PCBs to OH-PCBs have been published (Safe, 2000; Letcher *et al.*, 2000). In general, OH-PCBs are excreted but some may also be retained in blood (Bergman *et al.*, 1994, Fängström *et al.*, 2002). Over 40 OH-PCBs are present in human blood of which 39 have been identified (Hovander *et al.*, 2001). The OH-PCBs are present in blood at slightly lower concentrations than the parent PCB congeners (Sandau *et al.*, 2000; Sjödin *et al.*, 2000a), but at much higher concentrations in blood than in adipose tissues (Bergman *et al.*, 1994, Guvenius *et al.*, 2002). The lipid content of the blood compartment does not affect the retention of OH-PCBs since they are bound to plasma proteins (Brouwer *et al.*, 1998). The major OH-PCBs in human blood are metabolites of the highly chlorinated PCBs with the hydroxy group preferentially in the 4-position but occasionally in the 3-position (Bergman *et al.*, 1994, Sandau *et al.*, 2000, Sjödin *et al.*, 2000a, Hovander *et al.*, 2001). Levels of OH-PCBs in human blood samples from Northern Canada, Latvia, Sweden and the Faeroe Islands indicate that OH-PCB levels range between 10-20% of the parent PCB concentrations.

#### 6.3.2.2 Polybrominated diphenyl ethers (PBDEs)

As noted earlier, the plasma concentrations of PBDEs in humans from industrial countries do not appear to be decreasing. PBDEs are potential EDCs since both PBDE and their metabolites (OH-PBDEs) may interfere with the estrogen and/or thyroid system (Meerts *et al.*, 2001a). PBDEs are used as flame-retardants in high-impact polystyrene, flexible polyurethane foam, textile coatings, wire and cable insulation and electrical connectors. It has been reported that PBDEs may be released from television sets and computers and then absorbed into the lipid portions of the body (Sjödin *et al.*, 2000b, Sjödin *et al.*, 1999, Sjödin *et al.*, 2000a). Even though data are limited, it is clear that PBDEs are bioavailable and accumulate in human tissues. The half-lives of PBD congeners vary with the number of bromine atoms (Sjöden, 2000). The major

PBDE congener present in human blood or breast milk is 2,2',4,4'-tetrabromodiphenyl ether (Meironyté, 1999, Ryan, 2000, Strandman, 2000, Schröter-Kermani, 2000, Sjödin et al., 2000). Other PBDE congeners that have been detected, include the high molecular weight compound decabromodiphenyl ether (Sjödin et al., 1999; Sjödin et al., 2001). Higher levels of these PBDEs have been measured in personnel dismantling electronic products and in computer operators compared to controls (Jakobsson et al., 2002). Human exposure data, as determined in Canada, Germany, Latvia, Sweden and U.S.A., are summarized in Annex I (Table 23). There is some evidence that the levels in general are higher in North America than in other parts of the world (She et al., 2000; Päpke et al., 2001; Ryan and Patry, 2001), but further monitoring data is needed.

*Temporal trends:* In contrast to the chlorinated POPs, the concentration of the PBDEs in human breast milk samples from Sweden has doubled every 5 years from 1972 to 1997 as shown in Annex I (Figure 21) (Norén & Meironyté, 1999; Norén and Meironyté, 2000). Some preliminary time trend data from breast milk in Canada (Ryan et al., 2000) and in German blood (Schröter-Kermani et al., 2000), also indicates increased levels of PBDEs over time. In the last couple of years however the levels of one of the major PBDE constituents, BDE-47, have started to decrease in mothers milk (Gruenewald, 2002). Humans are exposed to PBDEs *via* contaminated food, such as, fatty fish and whale blubber (de Boer et al., 2000), *via* inhalation (Sjödin et al., 2000b).

PBDEs are metabolised to hydroxylated compounds and possibly sulphur containing metabolites (Hakk et al., 1999, Örn et al., 1998). A few OH-PBDEs have been reported as naturally occurring chemicals produced in marine algae and sponges (Gribble, 2000). Blood levels of OH-PBDEs are similar to those of the PBDEs (Asplund et al., 1999; Asplund et al., 2001; Hovander et al., 2001).

### 6.3.2.3 DDT

The manufacturing and use of a number of organochlorine pesticides, including DDT, have been banned or greatly restricted in many parts of the world since the 1970s. DDT, however, is still used in some developing countries, primarily for public health control of vector-borne diseases in the southern hemisphere. While the concentrations of DDT and the DDT metabolites,

DDE and methylsulfonyl-DDE are decreasing in the northern hemisphere, these compounds are still present at relatively high concentrations in some populations. Norén and Meironyte (2000) reported that levels of 4,4'-DDT and its metabolite, 4,4'-DDE, in Swedish human milk samples decreased dramatically during 1967-1997. The levels of dieldrin also decreased at the same rate. In contrast, measurements of DDT and its metabolites in 50 human milk samples collected in Mexico City in 1995 still showed relatively high levels (Torres Arreola et al., 1999). Concentrations up to 50,000 ng/g l.w. and more of DDT was also seen in blood samples (Annex I). Figure 6.6 summarizes concentration ranges of DDT, lindane, and hexachlorobenzene in different parts of the world. It is obvious from these data that levels are higher in geographical locations where certain chlorinated insecticides are still used, but in general low levels of these persistent compounds are found globally.

[insert Figure 6.6 here]

#### 6.3.2.4 Phthalates

Phthalates are diester derivatives of phthalic acid used primarily as plasticizers to make plastic products more flexible. Tetrabromophthalate diethylhexyl ester is used as a flame retardant. Certain plastics may contain up to 40% phthalate by weight. Consumer products containing plastics include: imitation leather, rainwear, footwear, upholstery, flooring, tablecloths, shower curtains, food packaging materials, children's toys, tubing and containers for blood transfusions and blood products. Since these plasticizers do not become a permanent (chemically bonded) part of the plastic matrix during the manufacturing process, they can migrate from the plastic product to environmental matrices under certain conditions. As a result, they have become ubiquitous in our environment, and people may be continually exposed to low levels of phthalate esters. Of particular recent concern is the potential for phthalate ester exposure to young children orally by chewing on toys, teething rings, and pacifiers containing these plasticizers (Steiner et al., 1999). Once in the body, phthalates are quickly metabolised to the corresponding monoester metabolite, which is then rapidly eliminated in the urine as its glucuronide conjugate or further metabolized. Concern has been raised about phthalate esters in relation to reproductive effects on adult males and development of male offspring (Chapters 3 and 5).



Few assessments of human exposure to phthalates have been reported. The major phthalates used in commerce are the diethyl; dibutyl; dicyclohexyl; butyl, benzyl; di-2-ethylhexyl; di-n-octyl; di-iso-nonyl; and di-isodecyl. Exposure to phthalates was measured (Blount et al., 2000a; Blount et al., 200b) in almost 300 urine specimens collected from adults in the U. S. NHANES III (1988-1994) survey. The phthalate mono-esters with the highest urinary levels were monoethyl phthalate (95<sup>th</sup> percentile, 3750 ppb, median, 305 ppb), monobutyl phthalate (95<sup>th</sup> percentile, 294 ppb, median, 41.0 ppb), and monobenzyl phthalate (95<sup>th</sup> percentile, 137 ppb, median 21.2 ppb) reflecting exposures to the parent compounds diethyl phthalate, dibutyl phthalate, and benzyl butyl phthalate. The authors speculated that metabolites of the more lipophilic phthalates, such as diethylhexyl phthalate, may be excreted *via* the bile and into the faeces.

#### 6.3.2.5 Atrazine

Atrazine is a member of the triazine herbicide family and has been widely used for weed control in agricultural crops. It is frequently found in surface water and ground water. Since it may be found in drinking water, its use has been banned or severely restricted in many countries. In the context of EDCs there is concern about atrazine related to the development of mammary tumours in exposed rats (See Chapter 3). Human exposure to atrazine has been assessed primarily by measurement of its metabolites in urine (Barr *et al.*, 1999, Catenacci *et al.*, 1993, Lucas *et al.*, 1993) or less frequently in blood. Limited data of internal atrazine exposure levels in human serum were indicated concentrations in the ppt and in urine at the ppb range (Barr *et al.*, 1999, Beeson *et al.*, 1999).

#### 6.3.2.6 Phytoestrogens

Seven phytoestrogens or their metabolites were measured in 200 human serum and urine specimens from adults participating in the NHANES III survey, but the results are not yet available. Preliminary data indicate that the levels were higher in the urine compared to the serum samples. The urine results did not differ greatly from the mean of the urinary levels reported in the literature from Western populations known to consume phytoestrogen supplements. Horn-Ross et al., (1997) examined urinary levels of several phytoestrogens in



samples from a multiethnic population of young women in the San Francisco Bay area. The highest urinary levels of coumestrol, lignans, enterodiols, and enterolactone were found in white women, and the lowest levels in Latin and African American women. Isoflavone levels were generally similar in all groups, but higher genistein levels were observed in Latin women.

Some mycotoxins (low molecular weight, cyclic metabolites produced by different species of fungi) have been shown to possess estrogenic potential. The primary route of general human exposure to these mycotoxins is *via* the food chain, but occupational exposures (e.g., processing peanuts and corn) may occur *via* inhalation. There is a paucity of data on human exposures to mycotoxins. The daily exposure of young Canadian children to zearalenone from food consumption has been estimated to be in the range of 0.05-0.10 µg/kg bw/day (Kuiper-Goodman et al., 1987).

#### 6.3.2.7 *Conclusions on human exposures*

There is still considerable uncertainty on associations between human health effects and exposure to EDCs. To resolve uncertainty, better exposure data must be generated. This is true for the general population as well as for the more susceptible subpopulations. Environmental human exposure to most EDCs occurs primarily *via* ingestion of food. Inhalation and dermal routes of exposure are generally not important. To date, exposure levels have primarily been measured in adults, and exposure(s) during critical development stages (e.g., foetus, infants and children) are urgently needed. Estimates of foetal exposures, are generally calculated from maternal levels, but these measurements may not accurately reflect exposure during critical stages of fetal development. Foetal samples such as cord blood and amniotic fluid, may also not reflect exposure during critical developmental periods. Meconium may be a more representative biological sample to assess foetal exposure. Additional data is needed on the distribution of EDCs within the body, on correlations among tissue levels, and excretory products. Computer generated exposure models need to be validated. Many EDCs occur in mixtures of related chemicals, posing particular measurement problems, and/or as part of complex mixtures. As yet, we know little about the relative importance in terms of hormonal activity of related chemicals and perhaps even less about complex mixtures. Mechanisms need to be developed to prioritize globally the most important potential EDCs. Mechanisms are also needed to promote better exchange of information with the intention of improving the comparability of national and regional monitoring programmes for EDCs.

## 6.4 Measurement of exposure to EDCs

Measurements of EDC residues may utilize special instruments (gas liquid or high performance liquid chromatography, mass spectrometry) similar to those for other environmental contaminants. However, methods which rely on biological activity, including some enzyme linked immunosorbent assays (ELISA) and assays that depend on protein-receptor binding, are finding increased utility particularly as screening tools; since the chemical nature of an endocrine disrupting sample may not be known, and biological activity may be the best (or only) indicator of the presence of EDCs. A tiered approach utilizing a combination of measurement methods is often desirable. In order to ensure high quality data, quality assurance (QA) procedures should be applied to all steps involved in sampling, analysis, data processing, and compilation of results (IPCS, 1992; 2000).

### 6.4.1 Sampling

Key issues in collecting samples to measure EDC exposures include:

1) representativeness of the samples; exposure may be assessed for a number of different reasons which may influence the choice of sampling locations or sampling matrices; 2) timing and frequency of sampling; collection of samples should address exposure of greatest concern (e.g., long term chronic exposure vs. intermittent short term exposure), patterns of contamination (e.g., continuous discharge vs. one-time accidental contamination), and address the endpoints of greatest concern; 3) selection of matrices; the choice of matrix is determined by factors such as relevance to route of exposure, ease and practicability of sampling, which analytes are to be assessed, and in some cases, ethical considerations; 4) statistics; appropriate statistical methods need to ensure the relevance of sampling (e.g., pooled vs. individual samples, numbers of samples from different locations/species); and 5) sample storage and preservation methods; special methods may need to be used to avoid contamination with other chemicals with potential EDC activity which are commonly found in sampling and laboratory equipment (e.g., plasticizers).

## **6.4.2 Analytical considerations**

### *6.4.2.1 Measuring specific chemicals*

Established analytical methods are readily available for most EDCs. Many countries have established regulatory authorities or requirements to provide standards for chemical analyses and methods for testing for residues in food or the environment. In developing countries, approaches for monitoring pesticide exposure are generally poorly developed and vary dramatically (Gonzales, 1999). A number of international organizations including the International Organisation for Standardization (ISO), the Association of Official Analytical Chemists International (AOACI), IUPAC, CODEX, and the OECD have initiatives to standardize methods and promote established protocols for producing acceptable data (Ambrus, 1999).

Most of the environmental monitoring studies of OCs have an unintentional bias because of the use of an electron capture detector that selectively detects halogen-containing chemicals from non-halogenated contaminants. Since these OCs also tend to be more persistent and bioaccumulate, scientists have focused disproportionately on these chemicals. A large array of other potential EDCs (e.g., alkylphenols, bisphenol A, 2,4,6-tribromophenol, tetrabromobisphenol A, and the OH-PCBs) may be overlooked because of lack of comparable analytical specificity. A number of phenolics are also naturally produced (Gribble, 2000) and these chemicals have received little attention in terms of broad EDC monitoring programs.

Multi-residue methods have the ability to test for a broad range of chemicals in a single sample and therefore are useful for screening purposes. There are multi-residue methods tailored for OCs, organophosphates, and dibenzodioxins/dibenzofurans. Other classes of chemicals (e.g., phenolics, steroids, carbamates) are analysed by class-specific or chemical-specific methods. The application of directed analysis permits more specificity, and is more likely to identify previously overlooked contaminants. Multicompound methods used in food testing could be adapted for use with other biological matrices (Seiber, 1999).

### *6.4.2.2 Identifying unknown samples*

Biological methods can be used as general screens to determine whether EDC-active chemicals are present or not in a given environmental sample, but they are limited in their ability

to identify specific chemicals. In order to verify the identity of the causative agent and to quantify the EDCs present, classical chemical methods should accompany biological techniques (Cech *et al.*, 1998). Chemical analyses of EDCs are similar to those employed for other organic residues. The preferred methods for EDCs are those that provide a maximum of structural information about the chemical at low concentrations (e.g., mass spectrometry) combined with biologically based analytical methods (see below).

#### 6.4.2.2.1 Biologically-Based Methods

The major biological methods currently available for detecting hormonally active substances are *in vitro* bioassays for assessing estrogenic or anti-estrogenic substances. Methods are also being developed to detect androgens and anti-androgens, thyroid active chemicals and chemicals that interfere with steroid biosynthesis and metabolism. International and national efforts are ongoing to develop standardized and validated test guidelines to identify, screen and detect endocrine disrupting chemicals (Zacharewski, 2000). *In vitro* bioassays have disadvantages including the inability to account adequately for *in situ* bioaccumulation, lack of metabolic capacity and the fact that they are generally specific for only one mechanism of action (e.g., receptor binding). A battery of screens must be employed if all possible mechanisms of endocrine disruption are to be addressed (Zacharewski, 2000).

Biologically based methods can be categorized as follows:

a) Receptor binding assays measure binding of agonists or antagonists to a specific cellular receptor. These methods rely on isolation of receptor ligands from test organisms (often mice or rats), which are then co-incubated with a high-affinity radioligand and with various concentrations of the test compound or mixture. Displacement of the radioligand is monitored against a known active compound, often estradiol. It does not take into account pharmacokinetics and metabolic effects, which would be part of an *in-vivo* interaction (Kramer *et al.*, 1997). The concentration range for effects for some of those tests can be extremely low (i.e., 0.06 ppt- to 0.2ppt) which is within the range of detectability of many modern analytical methods (Wooge *et al.*, 1992; Soto *et al.*, 1995).

b) Cell proliferation assays depend on the ability of estrogens to induce cellular proliferation in target organs such as rat pituitary cells and several human breast cancer cell lines

(e.g. MCF7 and T47-D cells). Cell proliferation is considered a hallmark of estrogen action (Hertz, 1985) and can be induced with very low levels of estrogenic substances (Soto et al., 1997).

c) Receptor-dependent gene expression assays measure the ability of a compound to stimulate a receptor dependent response in genes or induction of gene expression proteins in isolated cell lines. Various assays rely on use of transfected mammalian or yeast cells that trigger detection of a linked gene expression (e.g., production of an enzyme to degrade a sugar), the concentration of sugar remaining after the test is related to the potency of the chemical. These assays also do not address pharmacokinetics of the chemical. An *in-vivo* assay has been developed in Zebra fish where a receptor-dependent gene is stably introduced into the whole-organism (Legler, 2000). In this assay, a luciferase gene induction is utilised to detect transactivation of a transgenic estrogen receptor in whole-organism exposures to Zebrafish with measurable responses down to 0.1 nanomolar estradiol.

d) Immunoassays can detect the presence of a specific chemical at low, biologically relevant levels (Moye, 1999a). There are only a few classical chemical detection methods that are as effective as the ELISA methods in detecting chemicals below the ppb range. For classical methods to achieve comparable detection limits, extra efforts are needed to enhance the analyte's detectability through pre-concentration, derivatization or relying on ultra sensitive detection methods (e.g., electron capture) (Moye, 1996b). On the other hand, immunoassays may lack the specificity of chemical detection methods.

#### 6.4.2.2.2 Toxic identification and evaluation approach (TIE)

The TIE approach utilizes *in-vitro* bioassays in conjunction with chemical methods for identifying EDCs. The technique involves the use of toxicity-based fractionation procedures that can be utilized to identify endocrine active constituents in media such as water and food (Mount et al., 1988). The challenge is to combine an effective method for fractionating and isolating the compounds of interest with a bioassay that distinguishes endpoints relevant to the effect under examination. Bioassays, which have been used in TIE studies, include the DRCALUX (Chemical Activated LUciferase gene eXpression) for planar PCBs, dioxins and furans (Pauwels et al., 2000); the ER CALUX for ER agonists; recombinant yeast assays (Routledge et al., 1996) and reporter gene assays (Snyder et al., 2000). A recombinant yeast TIE system has been used to

assess sewage treatment effluents (Desbrow *et al.*, 1998) and a reporter gene assay for water samples (Snyder *et al.*, 2000). TIE methods are useful where some components of the mixture are clearly more hormonally active than others. TIE methods have been proposed for use on plasma to assess the relative contributions of endogenous *vs.* exogenous hormones (Sonnenschein *et al.*, 1995, Soto *et al.*, 1997).

### 6.4.3 Mixtures

Many potential endocrine disruptors exist as mixtures of related isomers and congeners (see Table 6.2). Individual chemicals within these mixtures may vary greatly in potency and may interact with each other in an unpredictable manner.

[insert Table 6.2 here]

Selective identification methods are available for related isomers, congeners and homologues, but these require much time and effort. Analytical methods for such mixtures ultimately require specific isomer standards for the correct quantification and identification. These are available for some (e.g. PCBs, PCDDs, PBDEs) but not for all classes of EDCs. While historical data generated for mixtures continues to be of value, efforts need to be made to relate this information to data now available on the individual components, so that previous exposures can be related to potential long-term effects. There is also need to continue to monitor for mixtures utilizing the best available technology so that individual isomer data as it becomes available can assist in reconstructing exposures.

#### 6.4.3.1 Chirality considerations

The concept of chirality relates to the spatial, 3-dimensional configuration of organic chemicals or the existence of non-superimposable mirror images (Kallenborn and Hühnerfuss, 2001). For chemicals that have asymmetric centers or isomers formed by hindered structural types (atropisomers), two different chiral forms exist for each center/type. As the number of centers increases, there is a geometric progression of possible configurational forms. Each possible configuration may have very specific biological effects that must be considered in any assessment of cause and effect. Some EDC effects are dependent on receptor binding depends on configurational parameters, and characterization of these chiral forms is critical.

Many PCB congeners exist as chiral pairs or atropisomers (Rodman *et al.*, 1991, Wong *et al.*, 2000) as do PCB methyl sulfones (Ellerichmann *et al.*, 1998) and many of the individual toxaphene isomers and chlordane components (Kallenborn and Hühnerfuss, 2001). Nonylphenol mixtures may contain as many as 120 separate structures with several likely to have chiral forms. Chiral selectivity for the endocrine effects of o,p'-DDT has recently been reported. (Wiese *et al.*, 1999) found that the R(-) enantiomeric form of o,p'-DDT displayed enhanced binding activity for estrogen receptor- $\alpha$ . The R(-) o,p'-DDT was also found to be significantly more potent than the S(+) form in estrogen dependent reporter assays (Wiese *et al.*, 1999). The enantiomer selective antiandrogen capacity of o,p'-methoxychlor was also observed (Wiese *et al.*, 1999). Chromatographic methods have been used to separate chiral forms of a number of pesticides (Buser *et al.*, 2000, Garrison *et al.*, 1996, Müller *et al.*, 1992; Ren *et al.*, 2000).

#### 6.4.3.2 TEF/TEQ Approaches

It has been suggested that approaches similar to the TEF/TEQ systems developed for dioxins and dioxin-like chemicals could be developed for EDCs (see section 6.3.2.1). This approach simplifies the handling of data on these compounds and gives a single measure of the toxicity of a sample. Various systems for weighting the amounts of individual congeners in a mixture have been used (NATO, 1989; WHO, 1997). A similar approach for EDCs might involve reporting activity in environmental samples in terms of estradiol equivalents. This approach has been used by Servos (2001) to report nonylphenol activity in environmental samples in terms of total estradiol equivalents.

#### 6.4.4 Quality assurance/quality control

The importance of QA/QC procedures in assessing exposure to chemicals has been reviewed (IPCS, 1993). Without adequate QA/QC procedures it is difficult to compare global monitoring data. Certain features are especially important for measuring EDC compounds; these include matrix spikes, precision measurements, spike recovery studies, detection limit determination, method validation, continuing calibration checks, quantitation with standard curves with known reproducibility, frequent documentation, adherence to Standard Operating Procedures (SOPs), instrument performance checks, standard expiration date adherence, intralaboratory comparisons when possible, analysis of reference standards, and frequent quality assurance audits (Fong, 1999). The application of QA/QC to biological measurements is equally

important. QA/QC procedures must address expected EDC concentration and biologic variation in the study performed (Bignert et al., 1994).

There is a large variation in detection limits for EDCs. Specific OCs have the lowest detection limits because of their responsiveness to electron-capture GC and GC/MS. Detection limits less than 0.001 ng/L in water are achievable by high sample volume preconcentration methods. Detection limits for organochlorine mixtures (PCBs, chlordane, toxaphene) are higher than for specific organochlorine compounds. For non-halogenated compounds (e.g., phthalate esters), detection limits are often 100 times higher than for halogenated EDCs. For the more polar EDCs, such as estradiol and nonylphenols, detection limits are also higher than for organohalogen. These differences in detection limits may introduce a bias toward generating more data on persistent EDCs. Newer, highly sensitive methods, which can detect a broader spectrum of EDCs, are being developed and are beginning to make an impact in the field of environmental exposure analysis for EDCs. When relating exposure to effects, detection limits need to be considered.

#### **6.4.5 Exposure models**

An exposure model is an empirical framework, which allows estimation of exposure parameters from available input data. Chemical release estimates, fate and transport modelling, and exposure potentials based on life habits can be employed to estimate exposure for wildlife and/or humans by way of air, food or water or in total. Models vary in their sophistication, geographic scope, data input needs and requisite computational power (Calimari, 1993; SETAC, 1994; Mackay, 2001, IPCS, 2000). Bioaccumulation potential can be estimated using existing models and these may provide estimates of external exposure concentrations without conducting lengthy monitoring and assessment programs (Sharp *et al.*, 2000). A widely accepted model for bioaccumulative exposure in humans is described in EPA's document on human health risk from dioxins due to combustion facilities (Sharp *et al.*, 2000, USEPA, 1994, USEPA, 1998, USEPA, 2000).

Calibrated exposure models for nonpersistent EDCs are not common, because there is currently little exposure data available to validate the models. Exposure estimates for these chemicals may need to be derived from surrogates. Under the European Existing Substances Regulation, modelling of potential exposure has been carried out for chemicals such as



nonylphenol, bisphenol A, and several phthalates. Models for estimating human exposure to pesticides through food consumption are described in many standard toxicology texts and have also been regularly updated for pesticides using market basket data and verification through monitoring programs (NRC, 1993; Olin, 1998; IPCS, 2000). These models would be applicable to non-pesticide EDCs, but have not been used to date probably because there is no regular monitoring system for most of these compounds.

The PCB food-chain model developed by (Thomann *et al.*, 1984) was used to project future residue levels in Hudson River and potential exposure to humans. Thomann *et al.* (1984) modelled feeding and gill absorption as the primary routes of uptake. For human consumption of foods from animal origin there is a model for 2,3,7,8-tetrachlorodibenzo *p*-dioxin transfers from incinerator emissions to humans (Fries *et al.*, 1999). Current models based on adult dosing are inadequate for estimating exposures *in utero* or neonatally. The present models need to be validated by comparison to monitoring data before they can be applied more generally.

#### **6.4.6 Structure Activity Relationships**

Structure-activity methods can be used to estimate the potential EDC activity of untested chemicals. So far, most progress has been made with structures which bind to the estrogen receptor (USEPA, 2001). If the bioassay test is based upon binding at the subcellular level and the active proteins at the binding site are fairly well characterized, then SAR approaches might work. An extensive study of ER binding affinity of a wide variety of steroidal and non-steroidal ligands was conducted by Waller *et al.* (1996). Fifty-five compounds were compared based on their steric and electrostatic properties. DES, selected estrogens, androgens, PCBs, organochlorine insecticides, phthalates and the hydroxylated metabolites of these compounds were all related to binding affinity in a statistically robust and internally consistent manner. The predictive limitations to this approach were due to the inconsistencies in the *in vitro* versus the *in vivo* systems used to generate the data (Jobling, 1998).

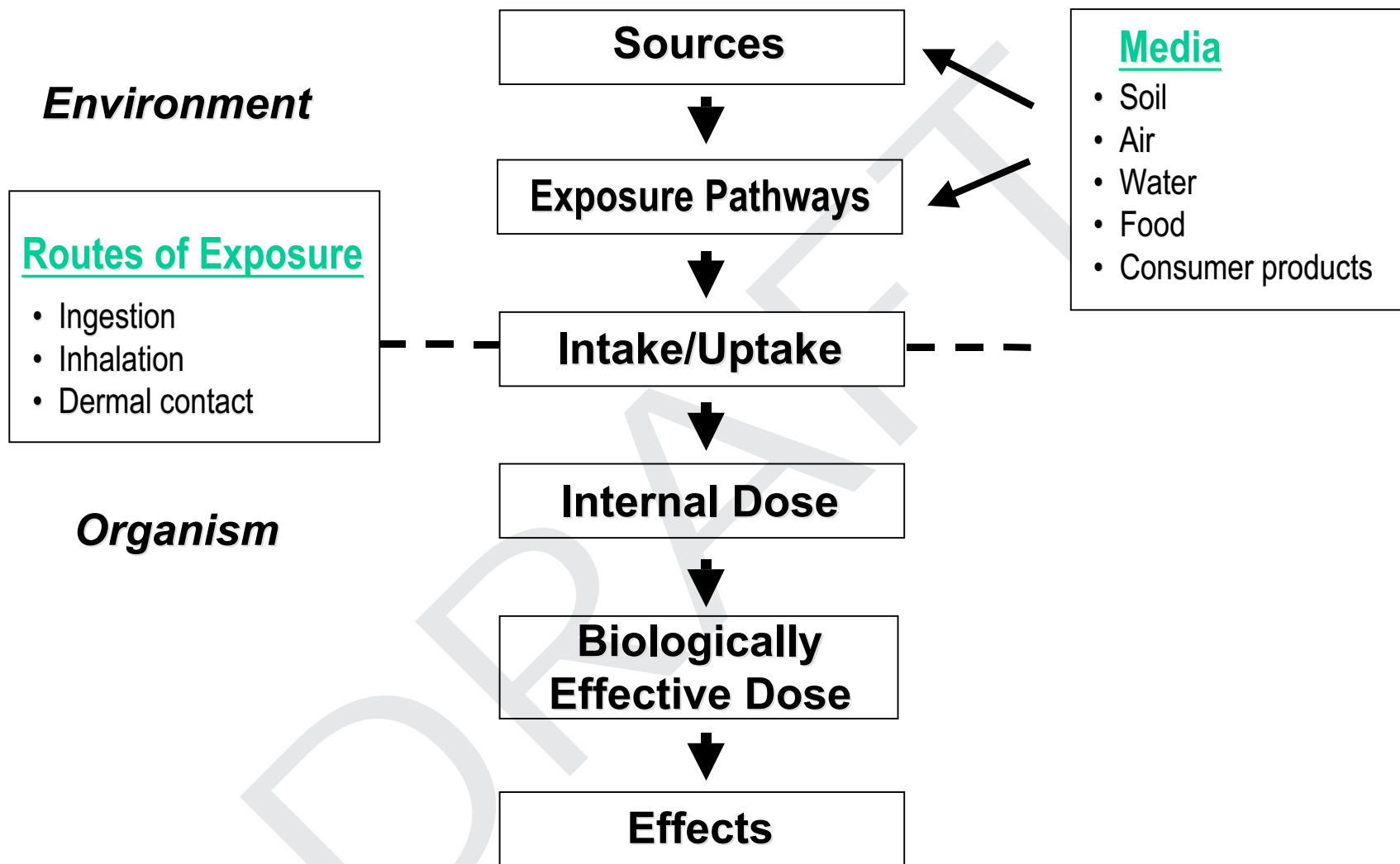
SAR can assist in identifying structural features common to a certain mode of action. The similarity of the structure of *o,p'*-DDT to DES was noted by (Bitman *et al.*, 1968) when they reported the estrogenic activity of *o,p'*-DDT in animals. Most environmental estrogens possess a *para*-substituted phenolic group (Jordan *et al.*, 1985). The presence of more than one phenolic group renders the compound more estrogenic (e.g., methoxychlor metabolites and diphenolic

isoflavonoids). Structural rigidity also appears to be a predictor of estrogenic potency because of improved receptor binding. Conformationally restricted PCBs have a structure analogous to steroids (McKinney et al., 1994). Dodge (1998) reported that quantitative structure-activity studies for PCBs indicate that the electron density of the aromatic rings in PCB molecules correlate with binding affinity to the ER. Hydroxyl substitution on the phenyl ring of the PCB also provides stronger binding affinity to the ER (Korach et al., 1988). The concept of conformational restriction helps explain the fact that the *o,p'* isomer of DDT is estrogenic while the *p,p'*-DDT is much weaker.

Ashby (1998) suggested that different structure activity relationships (SARs) will be required for each mechanism of endocrine disruption. Currently, most SARs are based on ER interaction that relate to the chemical structure of estradiol ( $E_2$ ), and predictability is poor for structurally remote analogs of  $E_2$  (e.g., kepone and dieldrin) or of testosterone (e.g. vinclozolin). Another drawback to the SAR approach is its failure to account for metabolic alterations that affect the activity of parent molecules. For example, s-methoprene becomes estrogenic after photolysis, forming a species that binds to the retinoic acid receptor (La Clair *et al.*, 1998). A similar situation exist with vinclozolin where *in vivo* activation produces metabolites that have androgen activity that would be missed with an *in vitro* test (Kelce et al. 1994 in (Jobling, 1998).

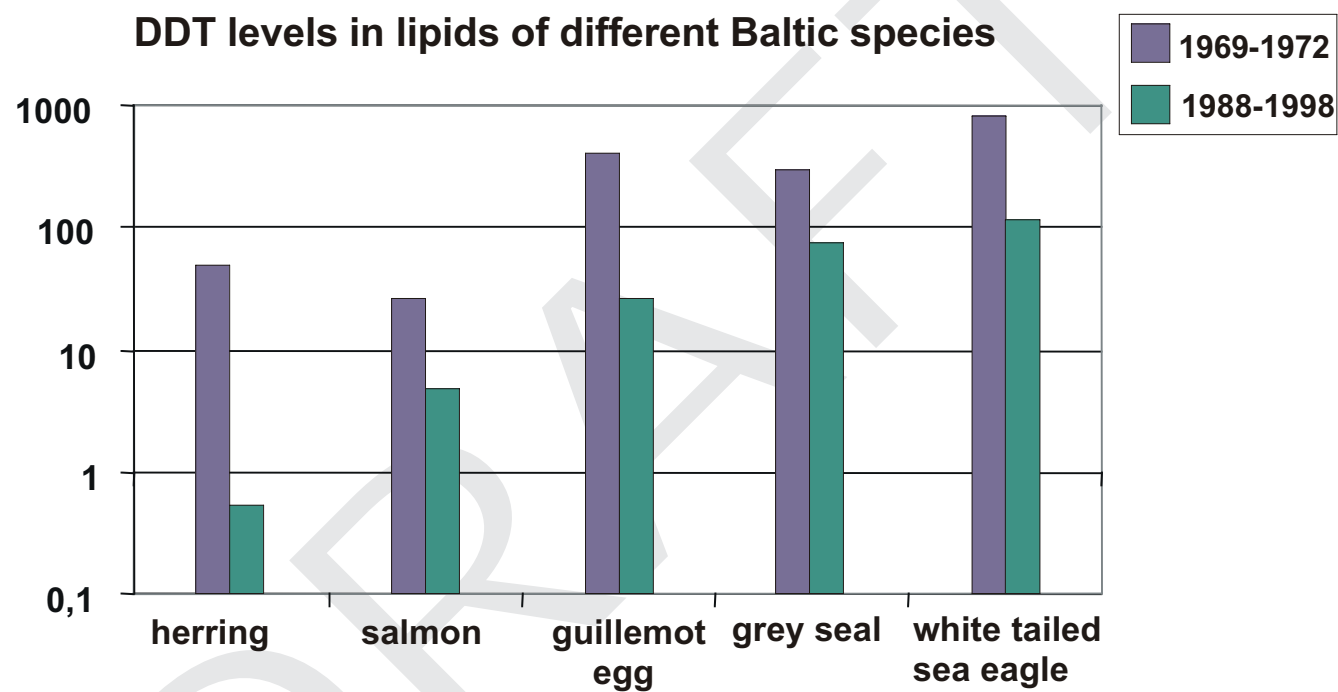
## 6.5 Summary

This chapter has illustrated the complexity and special problems related to measuring exposure to EDCs in wildlife and humans. The data clearly show that exposure to EDCs has occurred in wildlife species and human populations. However, except in isolated cases, data is not available to demonstrate specific associations between exposure and an endocrine mediated adverse effect. Most exposure data focuses on POPs in Europe and North America. Comparable data sets are not available for other non-persistent EDCs in other geographical regions, making it difficult to make a truly global assessment. Existing exposure data sets relate primarily to external exposures (air, food, water) rather than internal exposure (blood, tissue) with the exception of POPs in breast milk and some human blood concentration data. It is unrealistic to monitor all potential EDCs in all species and matrices on a global scale. International, coordinated efforts and mechanisms need to be established to prioritize monitoring and collection of exposure data, and to ensure comparability of data.



**Figure 6.1 Pathways of Exposure and Effects**

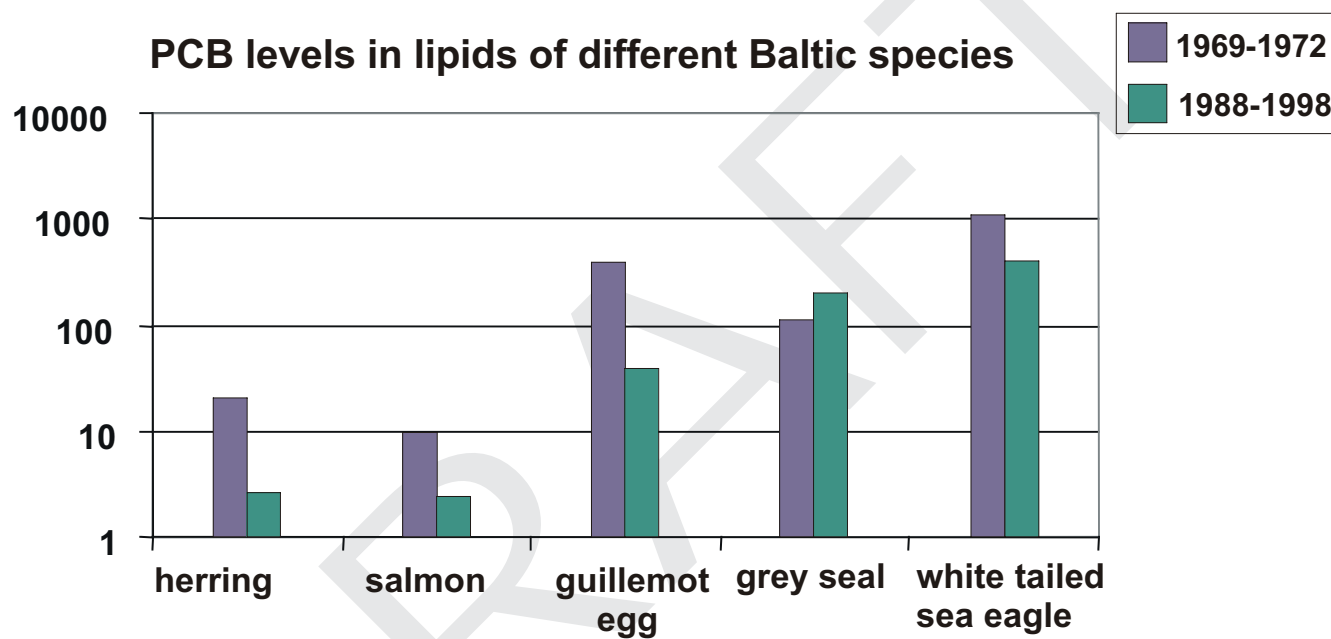
[\[back to text\]](#)



**Figure 6.2**

Representative concentrations of total DDT ( $\mu\text{g/g}$  lipid weight) in selected Baltic species from samples collected during 1969-1972 and 1988-1998. (For more details see Annex I – Table 1). All concentrations are log transformed.

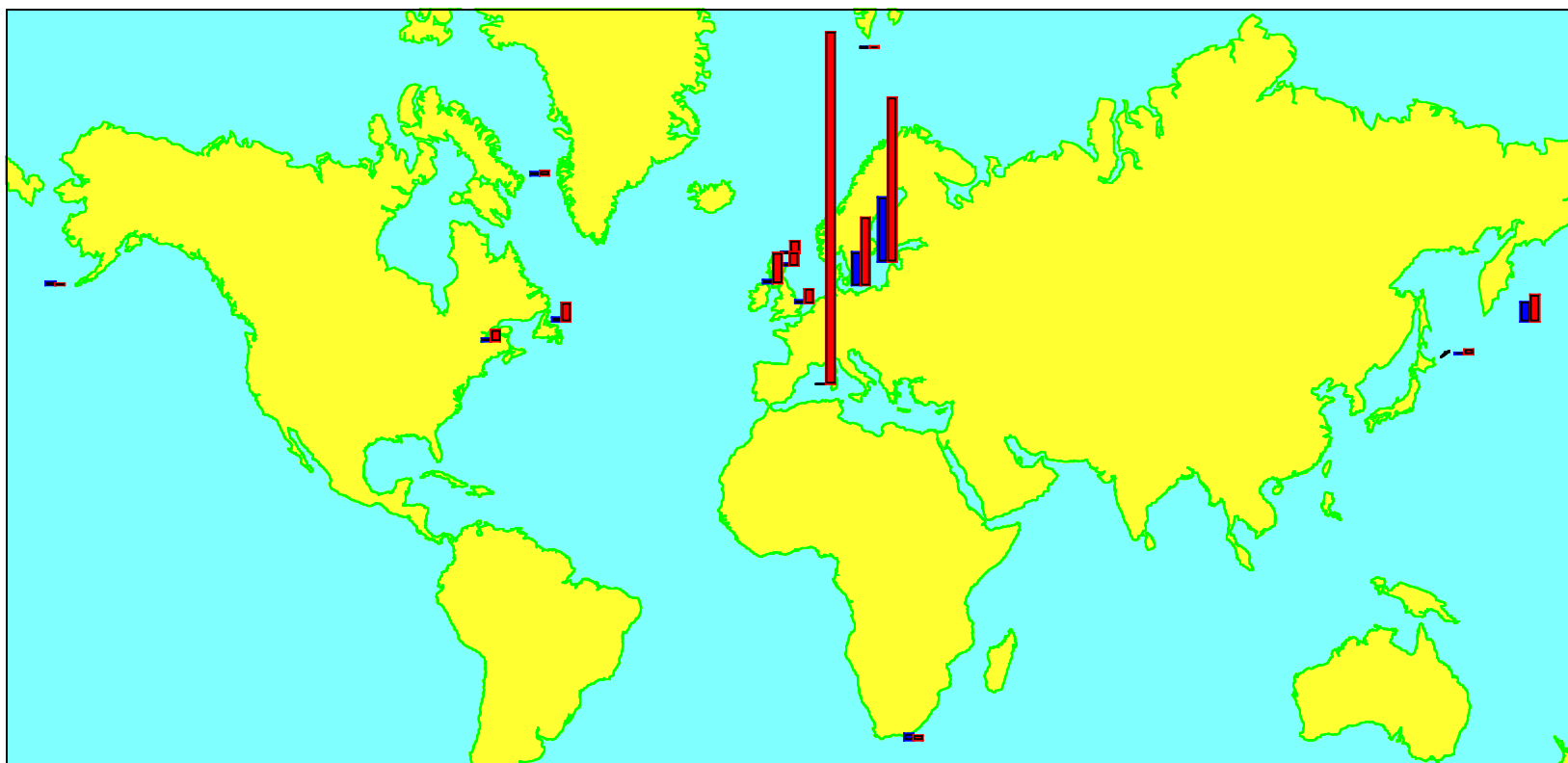
[\[back to text\]](#)



**Figure 6.3**

Representative concentrations total PCB ( $\mu\text{g/g}$  lipid weight) in selected Baltic species collected from samples During 1969-1972 and 1988-1998. All concentrations are log transformed. For more details see Annex I – Table 2).

[\[back to text\]](#)



**Figure 6.4**

Concentration range of total DDT (blue bars) and total PCB (red bars) ( $\mu\text{g/g}$  lipid w.) in marine fish-eating mammals from various part of the world. Highest bar =  $300 \mu\text{g/g}$  lipid weight. (For further details see Annex I – Table 16).

[\[back to text\]](#)

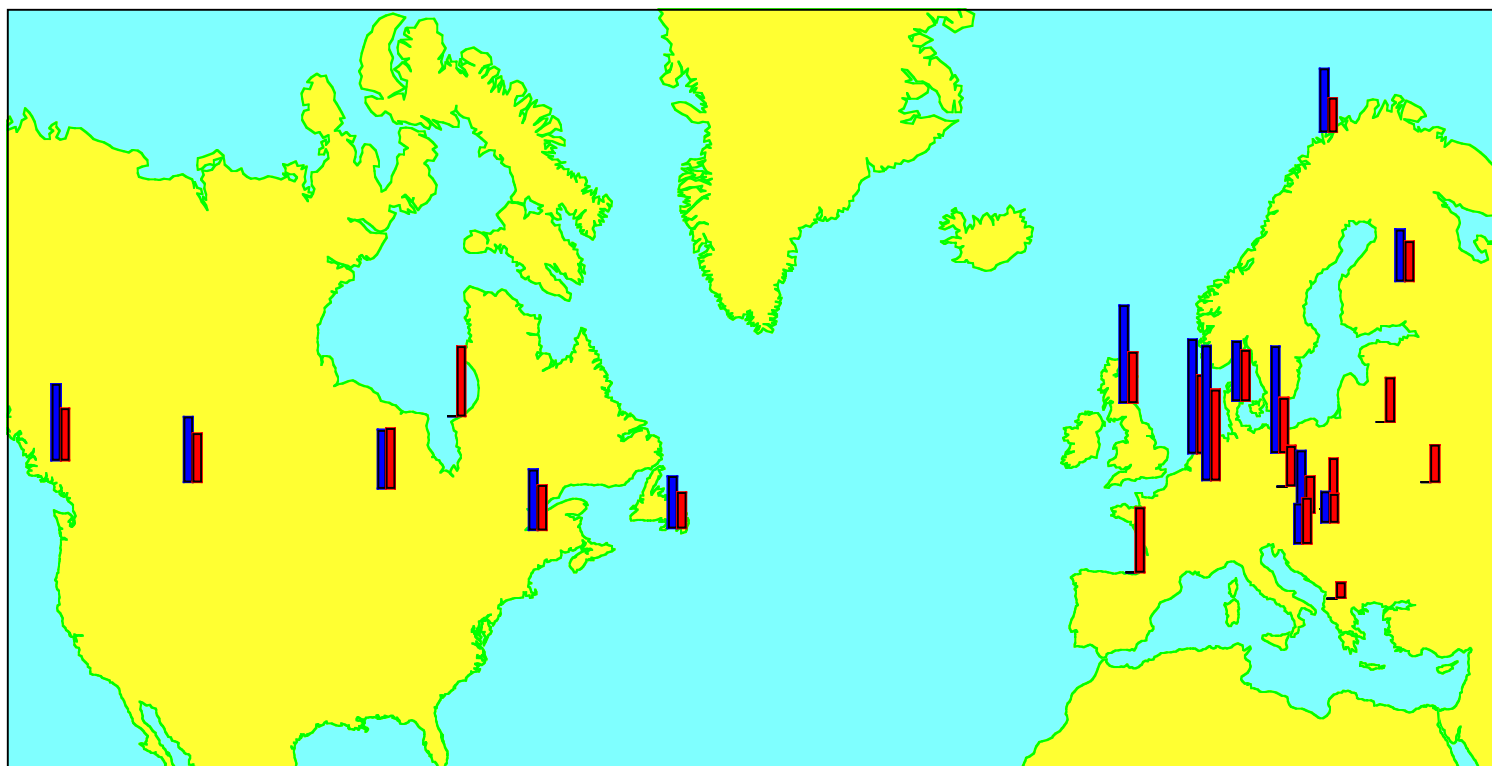


Figure 6.5

TEQs (pg/g lipid weight) and PCB concentrations (ng/g lipid weight) in human milk from 18 countries around the world.  
TEQs 1987/88 = blue bars and 1993/94 = red bars; highest bar = 40 pg/g TEQ lipid weight

[\[back to text\]](#)



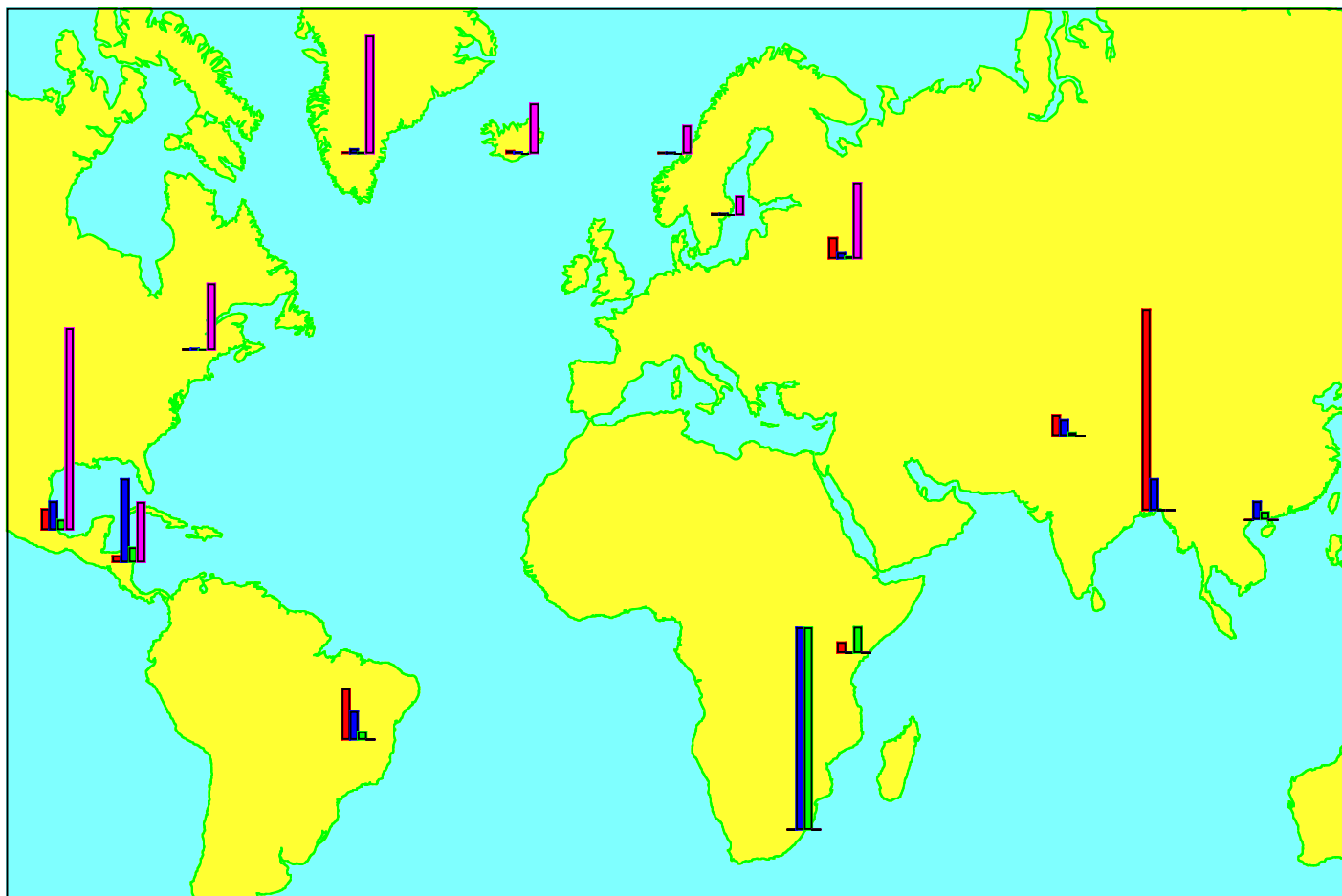


Figure 6.6

DDT and other pesticides in humans around the world

Red bars=b-HCH, blue=DDE, green=DDT, purple bars = HCB

[\[back to text\]](#)

**Table 6.1**

**Examples of Persistent EDCs in Wildlife in Three Geographic Regions**

	Baltic Sea	Great Lakes (USA)	Arctic
Environment	Marine enclosure, little clean water dilution; strong halocline. Temperate climate.	Connected freshwater lakes. Temperate climate.	Dramatic seasonal differences. Polar, seas and lakes covered by ice for much of the year.
Chemicals	DDT, PCBs, HCB, PCDDs, PCDFs, PBDEs, HCHs	DDT PCBs, PCDDs, PCDFs, PBDEs	DDT PCBs (inc OH-PCBs), PCDDs PCDFs
Sources	Highly industrialized communities within drainage areas.	Some areas of shoreline highly industrialized.	Highly industrialized areas on periphery. Long range transport for some pollutants.
Examples of species affected	<i>Fish:</i> Salmon <i>Birds:</i> White tailed sea eagle Guillimot Razorbill <i>Mammals:</i> Grey seal Otter Mink	<i>Fish:</i> Lake trout Chinook salmon <i>Birds:</i> Herring gulls Forster's terns Double crested cormorants <i>Mammals:</i> Mink	<i>Fish:</i> Arctic char <i>Birds:</i> Glaucous gull Thick-billed murre Puffin White tailed eagle <i>Mammals:</i> Mink Otter Polar bear Ringed seal

[\[back to text\]](#)

**Table 6.2**

**Common Environmental Mixtures of EDCs  
(their possible Component Numbers, Chiral Abundances and Commercial Standard Availability)**

Compound Name	Likely number of Components	Number of identified Components	Possible Chiral Forms	Commercial Standards Available	
				Components	Chiral Forms
Tech-DDT	6	6	2	6	2
Tech-chlordane	7 major (103) (>120 w minor)	~120 (104) (more when weathered)	4 major (~60 minor)	4	3 (major components)
Toxaphene	>200 (105)	13 (106)	3 identified (97% proposed) (106)	9	None
PCBs	209	209	19 (atropisomers)	209	None
Nonylphenol	~40 (107)	23 (108)	>30 (90)	None	None
PCDDs	19	19	0	19	None
PCDFs	16	16	0	16	None
Polybrominated diphenyl ethers	209	~15 (81)	0	41	None
Phthalates	18 (110)	18	2	7	None
Hexachlorocyclohexane (HCHs)	4	4	1	4	1
Endosulfan	2	2 (112)	2	2	2

[\[back to text\]](#)